

Pub #

Session Information

PRE-CONFERENCE EVENT: Endocrine Trainee Day - Class of 2011 (8:00 AM - 5:15 PM)

Title

Endocrine Trainee Day - Class of 2011

Author String

Body

Co-sponsored by The Endocrine Society & Women in Endocrinology; supported by Amgen, Endo Pharmaceuticals, and Pfizer, Inc.

Pub #

Session Information

PRE-CONFERENCE EVENT: Thyroid Workshop Advanced Course Lecture (9:00 AM - 11:30 AM)

Title

Thyroid Workshop Advanced Course Lecture

Author String

Body

Registration and fee required

Pub #

Session Information

PRE-CONFERENCE EVENT: Diabetes Diagnosis and Management Workshop (9:30 AM - 5:30 PM)

Title

Diabetes Diagnosis and Management Workshop

Author String

Body

Registration and fee required. Daiichi Sankyo, Inc., Lilly USA, LLC and Merck & Co., Inc.

Pub #

Session Information

PRE-CONFERENCE EVENT: Thyroid Hands-on Workshop - Advanced (11:30 AM - 1:30 PM)

Title

Thyroid Hands-on Workshop - Advanced

Author String

Body

Registration and fee required

Pub #

Session Information

PRE-CONFERENCE EVENT: Thyroid Workshop Beginner Course Lecture (1:30 PM - 3:45 PM)

Title

Thyroid Workshop Beginner Course Lecture

Author String

Body

Registration and fee required

Pub #

Session Information

PRE-CONFERENCE EVENT: Thyroid Hands-on Workshop - Beginner (3:45 PM - 6:30 PM)

Title

Thyroid Hands-on Workshop - Beginner

Author String

Body

Registration and fee required

Pub #

Session Information

PRE-CONFERENCE EVENT: Trainee Reception (5:30 PM - 7:00 PM)

Title

Trainee Reception

Author String

Body

Supported by Novo Nordisk, Inc.

Pub #

Session Information

CMES SYMPOSIA: Dual Perspectives in Diabetic Care: The Intersection of Endocrinology and Cardiology
(5:30 PM - 8:30 PM)

Title

Dual Perspectives in Diabetic Care: The Intersection of Endocrinology and Cardiology

Author String

Body

Organized by The American College of Cardiology Foundation and The Endocrine Society; supported by
Abbott Vascular, Genentech, Inc., Lilly USA LLC and sanofi-aventis US

Pub #

Session Information

PRE-CONFERENCE EVENT: Corporate Liaison Board Forum - Nuclear Receptors: From Bench to Bedside
(6:30 PM - 9:30 PM)

Title

Corporate Liaison Board Forum - Nuclear Receptors: From Bench to Bedside

Author String

Body

The Endocrine Society's Corporate Liaison Board

Pub #	L1-1
Session Information	PLENARY: TRANSLATIONAL - Presidential Address (7:45 AM - 9:15 AM)
Title	Presidential Address
Author String	KE Mayo Northwestern University, Evanston, IL
Body	Disclosure Incomplete: KEM

Pub # L1-2

Session Information PLENARY: TRANSLATIONAL - Presidential Plenary Lecture: The Nuclear Receptor Superfamily: How Physiology Rises out of the Genome (7:45 AM - 9:15 AM)

Title Presidential Plenary Lecture: The Nuclear Receptor Superfamily: How Physiology Rises out of the Genome

Author String RM Evans
Salk Institute/Howard Hughes Medical Institute, San Diego, CA

Body Nuclear receptors are ligand dependent transcription factors that play fundamental roles in organ physiology by regulating the activity of complex gene networks. They act through a common mechanistic template by recruiting chromatin modifying factors including repressors and activators and use this shared template to achieve genomic integration. Through this process the 48 human receptors control metabolic homeostasis, inflammation, reproduction, repair and cell growth. They are regulatory targets for classic nuclear active hormonal lipids including steroids, retinoids, vitamin D and thyroid hormone. The orphan branch of the family are controlled by non-classic nuclear hormonal lipids such as fatty acids, bile acids, cholesterol and lipophilic xenobiotic compounds.

The presentation will address key aspects as to how dynamic regulation of complex genomic networks is achieved in the context of circadian rhythm, diabetes, inflammation and cancer. In particular, nuclear receptor regulation of the balance between glycolytic and oxidative metabolism plays a key role in obesity, a primary risk factor for insulin resistance, hyperlipidemia, hypertension and heart disease. Exercise is a known beneficial factor in many diseases, and we have discovered that certain nuclear receptors active drugs can confer the benefits of exercise even in sedentary animals. The use of these 'exercise mimetics' to treat cardiovascular disease, frailty and insulin resistance will be discussed.

Nothing to Disclose: RME

Pub #	L1-3
Session Information	PLENARY: TRANSLATIONAL - Presidential Plenary Lecture: Nuclear Receptor Coactivators: Masters of Physiology & Disease (7:45 AM - 9:15 AM)
Title	Presidential Plenary Lecture: Nuclear Receptor Coactivators: Masters of Physiology & Disease
Author String	BW O'Malley Baylor College of Medicine, Houston, TX
Body	Nothing to Disclose: BWO

Pub #	M1
Session Information	MEET-THE-PROFESSOR: CLINICAL - Delayed Puberty (8:30 AM - 9:15 AM)
Title	Delayed Puberty
Author String	PB Kaplowitz Children's National Medical Center, Washington, DC
Body	Nothing to Disclose: PBK

Pub # M2
Session Information MEET-THE-PROFESSOR: CLINICAL - Difficult Diabetes Cases (8:30 AM - 9:15 AM)
Title Difficult Diabetes Cases
Author String GT McMahon
Brigham & Women's Hospital, Boston, MA
Body Session supported by: Lilly USA, LLC and Merck & Co., Inc.
Nothing to Disclose: GTM

Pub # M3

Session Information MEET-THE-PROFESSOR: CLINICAL - Drug Therapies for Osteoporosis: When, Which & How Long To Use? (8:30 AM - 9:15 AM)

Title Drug Therapies for Osteoporosis: When, Which & How Long To Use?

Author String AJ De Santis
University of Washington, Seattle, WA

Body The armamentarium of available and effective therapies to reduce osteoporotic fractures has become vast. This Meet the Professor session will discuss strategies to identify patients at risk for osteoporotic fractures in whom drug therapy would be indicated. Through the use of patient based case scenarios, we will discuss optimal treatment choice and duration. Special attention will be given to the growing concern regarding side effects of the most widely used class of osteoporosis medication, the bisphosphonates.

Session supported by: Amgen

Nothing to Disclose: AJDS

Pub #	M4
Session Information	MEET-THE-PROFESSOR: CLINICAL - Laboratory & Radiologic Evaluation of the Adrenal Incidentaloma (8:30 AM - 9:15 AM)
Title	Laboratory & Radiologic Evaluation of the Adrenal Incidentaloma
Author String	AH Hamrahian Cleveland Clinic Foundation, Chagrin Falls, OH
Body	Nothing to Disclose: AHH

Pub #	M5
Session Information	MEET-THE-PROFESSOR: CLINICAL - Management of Hypertriglyceridemia (8:30 AM - 9:15 AM)
Title	Management of Hypertriglyceridemia
Author String	J Brunzell University of Washington, Seattle, WA
Body	Nothing to Disclose: JB

Pub # S1-1

Session Information SYMPOSIUM SESSION: BASIC - Functional Relevance of G Protein-Coupled Receptor Dimers/Oligomers (9:30 AM - 11:00 AM)

Title Regulation of Opioid Receptor Heteromers by Morphine

Author String LA Devi
Mount Sinai School of Medicine, New York, NY

Body G protein-coupled receptor activity is regulated by a number of mechanisms. Among these, protein-protein interactions, and in particular interactions with other receptors, a phenomenon called receptor heteromerization, have been shown to play an important role in modulating receptor function. Emerging studies are revealing that heteromerization leads to activation of distinct signaling pathways, thus adding a new dimension to the regulation of receptor physiology. We have previously reported that opioid receptors can dimerize with each other as well as with other family A G protein-coupled receptors and that resulting heteromers exhibit novel pharmacological and functional properties. Since the majority of these studies were carried out with recombinant systems, we sought to generate reagents that would allow characterization of heteromers in endogenous tissue. Towards this end, we generated heteromer-selective antibodies to a number of receptor pairs including mu-delta opioid receptors. These antibodies are heteromer-selective since they recognize epitopes only in membranes from tissues of wild type animals (and not of animals lacking either mu or delta receptors); this supports the existence of receptor heteromers in vivo. We also find that these antibodies selectively block heteromer-mediated signaling. Using these antibodies we examined the regulation of heteromer levels under a variety of conditions that recapitulate pathological states. We find that the abundance of mu-delta heteromers is enhanced by chronic morphine treatment in areas of the central nervous system involved in pain perception. Both exogenous chaperones such as morphine and endogenous chaperones such as the receptor transport protein-4 are able to enhance heteromer abundance. Finally, the up-regulated mu-delta heteromer activates a distinct signaling pathway as compared to that activated by mu or delta receptors showcasing the mu-delta heteromer as a unique target for novel therapeutics in the treatment of chronic pain and addiction.

Sources of Research Support: NIH grants, DA 08863 and DA019521 (to L.A.D.).

Nothing to Disclose: LAD

Pub # S1-2

Session Information SYMPOSIUM SESSION: BASIC - Functional Relevance of G Protein-Coupled Receptor Dimers/Oligomers (9:30 AM - 11:00 AM)

Title Time-Resolved FRET between G Protein-Coupled Receptor Ligands Reveals Oligomers in Native Tissues

Author String T Durroux
Institute de Genomique Fonctionnelle, Montpellier, France

Body G protein-coupled receptors (GPCRs) have the propensity to dimerize when express in cell lines and these dimeric complexes have been proposed to play critical roles in cell signaling. However, their physiological impact remained elusive since no proof of the direct interaction of receptors in a native context had been provided. To demonstrate receptor proximity, the experiments based on an energy transfer between a donor and an acceptor are probably those of the most relevant since the transfer efficacy is dependent on donor-acceptor distance. However, one of the most difficult but also crucial steps is to label receptors in native tissue without modifying its sequence or its level of expression.

We have developed a new strategy based on time-resolved FRET and the use of lanthanide cryptate derivatized ligands. On the one hand, time-resolved FRET has been reported to exhibit very high signal-to-noise ratio because of the fluorescent properties of lanthanides. On the other hand, indirect but specific and efficient receptor labeling is achievable with fluorescent ligands. Specific FRET signals have been measured for the oxytocin, vasopressin V1a and V2, and dopamine D2 receptors, consistent with their dimeric nature in transfected cells. Interestingly, we have observed that fluorescent agonists have led to smaller FRET signals than antagonists. These results, in accordance with the binding data, strongly suggest that receptor dimer can be labeled by two antagonists on the same complex leading to a significant FRET signal while binding of two agonists is more difficult, inducing a smaller FRET signal. Finally, the strategy has been successfully applied on mammary gland preparation of lactating rats, demonstrating the presence of oxytocin dimers in a native context (Albizu et al, 2010) and therefore the existence of dimers in native tissues.

Albizu et al., Nat. Chem. Biol. 2010 6: 587-94.

Nothing to Disclose: TD

Pub # S1-3

Session Information SYMPOSIUM SESSION: BASIC - Functional Relevance of G Protein-Coupled Receptor Dimers/Oligomers (9:30 AM - 11:00 AM)

Title Dopamine Receptor Heteromerization Generates Novel Complexes That Stimulate Calcium Signaling in the Brain

Author String SR George
University of Toronto, Toronto, Canada

Body Dopamine receptors are involved in mediating the many actions of dopamine including learning and memory, reinforcement and reward, locomotion, regulation of hypothalamic and pituitary hormones. The five dopamine receptors have primarily been characterized through their modulation of adenylyl cyclase activity. We have shown that the most abundant of dopamine receptors, the D1 and D2 receptors heterooligomerize to form a novel signaling complex that signals through Gq protein, phospholipase C and inositol phosphate receptor linked intracellular calcium release, quite distinct from the signaling of D1 or D2 homooligomers. This novel D1-D2 signaling complex, shown for the first time to occur in native neurons, has been shown to exist in brain and cultured neurons, through innovative confocal FRET methodology and generates a calcium signal that leads to a cascade involving calcium calmodulin kinase II activation and increased expression of brain derived neurotrophic factor. The D5 receptor, which is much less abundantly expressed in brain, can couple to Gq to activate calcium release, which in turn activates store operated calcium channels to induce an influx of calcium. Heteromerization of the D5 receptor with the D2 receptor modulates the D5-induced calcium signal to attenuate it significantly. These novel dopamine receptor heteromeric complexes have novel signaling and functional capabilities that broaden the repertoire of signaling pathways available to the individual G protein coupled dopamine receptors.

Sources of Research Support: NIH National Institute on Drug Abuse and the Canada Research Chairs Program.

Nothing to Disclose: SRG

Pub # S5-1

Session Information SYMPOSIUM SESSION: BASIC - Sick of Being Stressed: Neuroinflammation & the Hypothalamic-Pituitary-Adrenal Axis (9:30 AM - 11:00 AM)

Title Dynamics of the Central Nervous System Inflammatory Response to Stressors

Author String T Reyes
University of Pennsylvania, School of Medicine, Philadelphia, PA

Body Hypothalamic-pituitary-adrenal (HPA) activation is a critical response to systemic infection. Engagement of multiple cell types that compose the cerebral vasculature represents an important mechanism that links peripheral immune activation to central responses. Data will be presented that describe the role of prostaglandin synthesis in distinct vascular cell types. These responses vary in response to different systemic immune challenges, and vary in their importance to the physiological/behavioral responses. Additionally, data will be presented that highlight the importance of sometimes overlooked variables, including sex differences, differences across the lifespan, and the role of prenatal nutritional challenges.

Sources of Research Support: NIH MH087978; NIH MH091372; NIH DK064086.

Nothing to Disclose: TR

Pub # S5-2

Session Information SYMPOSIUM SESSION: BASIC - Sick of Being Stressed: Neuroinflammation & the Hypothalamic-Pituitary-Adrenal Axis (9:30 AM - 11:00 AM)

Title Neurocircuits Regulating Stress & Inflammation

Author String JP Herman
University of Cincinnati, Cincinnati, OH

Body Stress and inflammation are adaptive physiological reactions to adversity. Importantly, both classical 'stress' responses (i.e., glucocorticoids (GC) release) and immune responses are triggered by psychogenic stimuli. The ability of psychological experiences to drive neuroimmune, as well as neuroendocrine and autonomic responses, indicates that the former is an active participant in the physiological stress 'reaction'. Indeed, abnormal cytokine production is linked to stress-related diseases, e.g., depression. The neurocircuitry controlling psychogenic activation of the hypothalamo-pituitary-adrenocortical (HPA) axis has been intensively studied, and involves oligosynaptic links between limbic regions (such as prefrontal cortex, hippocampus and amygdala) and corticotropin releasing hormone neurons of the hypothalamic paraventricular nucleus (PVN). By comparison, brain pathways activating peripheral cytokine (e.g., IL-6) responses to psychogenic stress are poorly understood. Data from our group and others indicate that psychogenic stress triggers cytokine production within stress regulating brain regions (e.g. IL-1B in the hypothalamus), and that the magnocellular system of the PVN and SON may contribute to the peripheral increase in circulating cytokines (e.g. IL-6). Indeed, IL-6 magnocellular neurons are selectively recruited during psychogenic stress exposure. Notably, neuroimmune and neuroendocrine responses to psychogenic stress appear to require ascending input from the nucleus of the solitary tract (NTS). Stress-activated neurons of the NTS are innervated by prefrontal cortex and central amygdala, suggesting a means by which psychogenic stimuli may be translated into coordinated activation of endocrine, autonomic and immune effector systems. Thus, psychogenic activation of the immune system appears to be coordinated in tandem with so-called 'classical' stress effectors, and is likely to play an important role in both systemic stress adaptation and stress pathology.

Sources of Research Support: NIH grants MH049698 (JPH), MH069725 (JPH), MH069860 (JPH) and MH084515 (RJ).

Nothing to Disclose: JPH

Pub # S5-3

Session Information SYMPOSIUM SESSION: BASIC - Sick of Being Stressed: Neuroinflammation & the Hypothalamic-Pituitary-Adrenal Axis (9:30 AM - 11:00 AM)

Title Why Do We Feel Sick & Behave in a Sick Way When We Are Ill?

Author String RE Dantzer
University of Illinois, Urbana, IL

Body Anyone who has experienced a viral or bacterial infection knows very well the feelings of sickness, in the form of malaise, lassitude, fatigue, numbness, chills, muscle and joint aches, and reduced appetite. Because these symptoms are common, physicians tend to dismiss them as uncomfortable but essentially unhelpful aspects of the pathogen induced debilitation process with no benefit to the sufferer's well being. This view has turned out to be not only simplistic but incorrect. The psychological and behavioral components of sickness represent, together with the fever response and associated neuroendocrine changes, a highly organized strategy of the organism to fight infection. This strategy is referred to as sickness behavior. The development of sickness is triggered by the proinflammatory cytokines that are produced by activated cells of the innate immune system in contact with specific pathogen-associated molecular patterns. These cytokines include IL-1, IL-6, and TNF-alpha. The same cytokines are produced in the brain by macrophage-like cells including microglia in response to peripherally produced cytokines. They ultimately act on neurons to alter body metabolism and behavior. In the same way as inflammation normally resolves and leaves room for repair mechanisms, sickness behavior is normally followed by recovery. However, when the peripheral immune responses is too intense or lasts too long, the behavioral response to cytokines can become maladaptive and culminate in an episode of depression. The mechanisms of transition from sickness to depression have been studied both in the clinics in patients receiving chronic cytokine immunotherapy for the treatment of chemotherapy resistant tumors or hepatitis C virus, and at the bench, in animal models of inflammation-induced depression. The transition from sickness to depression is mediated by activation of the tryptophan-degrading enzyme, indoleamine 2,3 dioxygenase, which is the first and rate-limiting enzyme of the kynurenine metabolic pathway. Inhibition of this enzyme by pharmacological or genetic means abrogates the development of depression but does not affect sickness in response to inflammation. In contrast inhibition of the cytokine response abrogates both sickness and depression. The way this research ultimately impacts on our understanding of the occurrence and treatment of non-specific symptoms in physically ill patients and in the aging population will be discussed.

Sources of Research Support: NIH grants to Keith W. Kelley (R01 AG 029573 and R01 AG 029573-04S1) and Robert Dantzer (R01 MH 71349 and R01 MH 079829).

Disclosures: RED: Ad Hoc Consultant, Astra Zeneca, Bristol-Myers Squibb; Consultant, Lundbeck.

Pub # S2-1
Session Information SYMPOSIUM SESSION: BASIC - Mechanisms of Islet Cell Development (9:30 AM - 11:00 AM)
Title Using Stem Cells To Reconstruct a Pancreas
Author String DA Melton
Harvard University/Howard Hughes Medical Institute, Boston, MA
Body Session supported by: Lilly USA, LLC
Disclosure Incomplete: DAM

Pub # S2-2

Session Information SYMPOSIUM SESSION: BASIC - Mechanisms of Islet Cell Development (9:30 AM - 11:00 AM)

Title Postnatal Maturation of Beta Cell Function

Author String S Bonner-Weir
Harvard Medical School, Boston, MA

Body In vitro insulin-producing cells derived from human ES cells lack the glucose-responsive insulin secretion that is characteristic of a functional beta cell. Neonatal rodent islets also lack this glucose responsiveness and so serve as a model for the induction of the maturation process. Using laser capture microdissection (LCM) and Affymetrix microarrays, we excised beta cell-enriched cores of pancreatic islets from day 1 (P1) neonatal and young adult Sprague-Dawley rats and compared their gene expression profiles. We identified genes that were differentially expressed in neonatal P1 and adult beta cells that suggest that the known lack of glucose-induced insulin secretion of neonatal beta cell is due to a generalized immaturity of phenotype, ie that the specialized metabolism of the mature beta cell is lacking. Among the genes lower in the neonatal beta cells were key metabolic genes including mitochondrial shuttles, pyruvate carboxylase and carnitine palmitoyl transferase 2. Using qPCR on RNA from isolated neonatal (P2 until P28) and adult islets, we found 5 different patterns of islet gene expression with inflection points between P7 and 9 days and again about P15, times at which there are major plasma hormone (thyroid hormone, leptin, cortisterone and prolactin) and nutrient changes.

Additionally key beta cell transcription factors including glucose-responsive transcription factors Mafa, NeuroD1 and Pdx1 have very low expression in neonatal beta cells, being only about 10% that of the adult mRNA levels. By P7 Pdx1 and NeuroD1 no longer differ from adult but Mafa expression remained significantly lower than adult through P21. Adenoviral-mediated expression of Mafa in P2 rat islets induced glucose-stimulated insulin secretion (GSIS) almost to adult levels, with both the percentage of secreting beta cells and the amount of insulin secreted per beta cell increased. In contrast, similar adenoviral-mediated expression of Pdx1 had only modest effects on GSIS. So far, our studies show that T3 and possibly dexamethasone are physiological regulators that can induce similar increases in Mafa and GSIS and thus functional maturation.

In conclusion, in dissecting the mechanisms of functional maturation by which neonatal beta cells acquire glucose-responsive insulin secretion, we have found a coordinated gene expression program in which Mafa plays a crucial role and is regulated by physiological factors.

Session supported by: Lilly USA, LLC

Co:authors: A.Jermendy, C.Aguayo-Mazzucato, E.Toschi,A.Koh, A.Marinelarena, D.Sgroi, GC Weir, A.Sharma

Sources of Research Support: NIH grant DK66056; Graetz Fund

Nothing to Disclose: SB-W

Pub # S2-3
Session Information SYMPOSIUM SESSION: BASIC - Mechanisms of Islet Cell Development (9:30 AM - 11:00 AM)
Title Pancreatic Islets: Vascularization, Innervation & Regeneration
Author String AC Powers
Vanderbilt University Medical Center, Nashville, TN
Body Session supported by: Lilly USA, LLC
Nothing to Disclose: ACP

Pub # S3-1

Session Information SYMPOSIUM SESSION: BASIC - Novel Nuclear Receptor Modulators (9:30 AM - 11:00 AM)

Title Estrogens of Unusual Structure Having Desirable Patterns of Selective Activity

Author String JA Katzenellenbogen
University of Illinois, Urbana, IL

Body The pharmacology of estrogen receptor ligands is rich and multifaceted, and it provides many possibilities for achieving desirable selective physiological and pharmacological effects. It has proved challenging, however, to reach optimal selectivities and also to understand the mechanistic basis for these selectivities. The tissue-selectivity that various estrogens exhibit can have its basis in a number of selective interactions: (a) differential binding affinity of ligands for the two estrogen receptor subtypes, ER-alpha and ER-beta, (b) differential coactivator interactions of the ER-ligand complexes, and (c) differential activation of nuclear vs. extranuclear ER signaling pathways. We have developed assays by which these different interactions can be probed conveniently and in a quantitative manner. We have also prepared ER ligands of diverse and unusual structures, and we have shown that upon binding, they induce novel conformations of the ERs. Some of these ligands demonstrate unusual, and highly desirable, patterns of biological selectivity in vivo, such as cardiovascular protection and neuroprotection without stimulation of reproductive tissues or breast cancer. These aspects will be discussed.

Sources of Research Support: NIH R37 DK015556 and P50 AT006268 (to J.A.K.); P50 AT006268 (to B.S.K.); R01 DK077085 (to K.W.N.); R01 CA052599 (to C.K.G.); R01 HD030276 (to P.W.S.); P01AG013918 (to S.C.M.).

Nothing to Disclose: JAK

Pub # S3-2

Session Information SYMPOSIUM SESSION: BASIC - Novel Nuclear Receptor Modulators (9:30 AM - 11:00 AM)

Title Cadmium as Estrogen Receptor Modulator

Author String MC Louie
Dominican University, San Rafael, CA

Body Metalloestrogens are a class of inorganic xenoestrogens that have the ability to mimic the effects of estrogen. Among the metalloestrogens, cadmium is best characterized and has been shown to play a significant role in estrogen receptor (ER)-regulated processes including gene expression and cell growth. The biological actions of estrogens are mediated by several receptors including ER α , ER β and the membrane form of ER, GPR30. Although the estrogen receptors appear to bind to a broad range of estrogenic compounds (i.e. phytoestrogens, antiestrogens, and metalloestrogens), individual differences in ligand specificity among the three receptors do exist, which leads us to question how cadmium functions to mediate the actions of these receptors. It has been well documented that cadmium functions as an endocrine disruptor to stimulate ER α activity and promote target gene expression (1-6). In addition to ligand binding, the activity of the estrogen receptor is also determined by the interaction and cross talk with other transcription factors and co-regulators. Specifically, cadmium has been shown to modulate the interaction of ER α with c-jun to regulate transcription. While the role of cadmium is well established in ER α -mediated signaling, lesser is known about its role in ER β and GPR30 regulated pathways. Although results from a recent study have suggested that cadmium may bind to GPR30 to promote the activation of the MAPK cascade leading to Erk-1/2 phosphorylation, further studies are necessary to better understand the role of cadmium in GPR30 and ER β signaling (7).

(1) Garcia-Morales et al., J Biol Chem 1994; 269(24): 16896-16901
(2) Antila et al., Sci Total Environ 1996; 186(3): 251-256 1996
(3) Stoica et al., Mol Endocrinol 2000; 14(4):545-553
(4) Johnson et al., Nat Med 2003; 9(8): 1081-1084
(5) Martin et al. Endocrinology 2003; 144(6): 2425-2436.
(6) Siewit et al., Mol Endocrinol 2010; 24(5):981-992
(7) Yu et al., Toxicology and Applied Pharmacology 2010; 245: 83-90

Sources of Research Support: NIH Grant 2R15CA121983-02, NIH Grant 1R15CA121983-01A1, Wendy Wil Case Cancer Fund awarded to MCL.

Nothing to Disclose: MCL

Pub # S3-3

Session Information SYMPOSIUM SESSION: BASIC - Novel Nuclear Receptor Modulators (9:30 AM - 11:00 AM)

Title Therapeutic Actions of Synthetic REV-ERB Ligands in Animal Models of Metabolic Disease

Author String TP Burris
The Scripps Research Institute, Jupiter, FL

Body REV-ERB α and REV-ERB β are nuclear receptors that were considered orphan members of the superfamily until only recently. We identified the porphyrin heme as a physiological ligand that binds directly to the ligand binding domains of both REV-ERBs and regulates their ability to recruit corepressor and thus their ability to silence gene transcription. These two receptors play an essential role in regulation of the cellular clock and the physiological circadian rhythm. They have also been demonstrated to play key roles in regulation of lipid and glucose metabolism. We have developed a series of potent and efficacious synthetic REV-ERB ligands, both agonists and antagonists, which modulate the activity of the receptors. We have also developed REV-ERB ligands that have significant in vivo exposure allowing us to examine the potential to target these receptors for the purpose of treating various diseases including metabolic disorders. In this presentation, I will describe our identification and characterization of these novel compounds as well as describe their utility in the treatment of metabolic disorders.

Sources of Research Support: DK080201, DK089984, MH092769.

Nothing to Disclose: TPB

Pub # S4-1

Session Information SYMPOSIUM SESSION: BASIC - Novel Signaling Mechanisms in Consumption: Food, Drugs & Drinking (9:30 AM - 11:00 AM)

Title Divergent Signaling Mechanisms of Angiotensin II in Thirst & Salt Intake

Author String D Daniels
University at Buffalo, State University of New York, Buffalo, NY

Body The potent stimulation of water and salt intake by angiotensin II (AngII) is a classic model of endocrine regulation of behavior. Although the dipsogenic property of AngII has been a topic of intense investigation since its discovery almost fifty years ago, our understanding of the roles that relevant intracellular signaling pathways play in water and salt intake is in its infancy. Data from our laboratory suggest that the diverse intracellular cascades stimulated by AngII have separate roles in water and salt intake. In these studies, we injected rats with Sar¹,Ile⁴,Ile⁸-AngII, a peptide analog of AngII that activates MAP kinase family members (ERK1/2) without concomitant IP₃ formation, and found that saline intake was stimulated, but AngII-induced water intake was inhibited. Subsequent studies found that pharmacological inhibition of PKC reduced water intake in response to AngII, but had no effect on AngII-induced salt intake. On the other hand, inhibition of MAP kinase decreased saline intake after injection of AngII, but did not affect water intake in the same test. Although these studies suggest that MAP kinase is not required for the water intake stimulated by AngII, additional studies in our laboratory suggest a critical role for MAP kinase in the behavioral desensitization (tachyphylaxis) of water intake observed after repeated injections of AngII. Specifically, Sar¹,Ile⁴,Ile⁸-AngII produced an AngII-like behavioral desensitization and inhibition of MAP kinase prevented desensitization normally observed after repeated treatment with AngII. Taken together, these studies reveal previously unappreciated roles for AngII-mediated intracellular signaling pathways that may have important clinical relevance and point to a potentially unique divergence in the behavioral relevance of intracellular signaling pathways.

Sources of Research Support: NIH Grants DK73800 and HL91911 awarded to DD.

Nothing to Disclose: DD

Pub # S4-2

Session Information SYMPOSIUM SESSION: BASIC - Novel Signaling Mechanisms in Consumption: Food, Drugs & Drinking (9:30 AM - 11:00 AM)

Title Functional Role of SOCS3 in Regulation of Hypothalamic Leptin Resistance & Long Term Energy Balance

Author String A Xu
University of California, San Francisco, CA

Body Suppressor of Cytokine Signaling 3 (Socs3) is a negative regulator of Signal Transducer and Activator of Transcription 3 (Stat3) signaling, and its expression is increased in the hypothalamus of animals that are fed with a high fat diet. To determine the functional consequence of Socs3 upregulation on leptin signaling and obesity, we have generated transgenic mice that specifically overexpress Socs3 in hypothalamic neurons expressing Proopiomelanocortin (Pomc) or Agouti-related peptide (AgRP). We show that mice with Socs3 upregulation in Pomc or AgRP neurons exhibit distinct phenotypes that resemble different aspects of metabolic disturbances observed in diet-induced obesity. Our results suggest that Socs3 upregulation in melanocortineric neurons exerts a cell type specific role in regulation of hypothalamic leptin resistance, long term energy balance and glucose homeostasis.

Sources of Research Support: In part by research grant from the American Diabetes Association (ADA 7-07-JF-68).

Nothing to Disclose: AX

Pub # S4-3

Session Information SYMPOSIUM SESSION: BASIC - Novel Signaling Mechanisms in Consumption: Food, Drugs & Drinking (9:30 AM - 11:00 AM)

Title Endocannabinoid Signaling & Energy Balance

Author String D Cota
Institut National de la Santé et de la Recherche Médicale, Bordeaux Cedex, France

Body The endocannabinoid system (ECS), which includes at least two cannabinoid (CB) receptors, specific endogenous ligands, called endocannabinoids, and their biosynthesis and degradation pathways, has been recently recognized to play an important role in energy balance, by affecting both central nervous system (CNS) and peripheral mechanisms regulating food intake, adiposity and peripheral metabolism. In obesity, the ECS is dysregulated and synthetic antagonists targeting CB1 receptors have shown potential for the treatment of this condition. This lecture will review the published evidence on the role of the ECS in energy balance. Data that we have recently generated will provide new insights on the role of this system in the context of food intake and body weight regulation and on its functional relationship with intracellular fuel sensing mechanisms that have been recently implicated in such regulation. Results that we have obtained, which suggest that circulating endocannabinoids might have a role in the regulation of human eating behavior, will be also discussed.

Taking into account that the ECS is being investigated as a target for the pharmacologic treatment of obesity and its metabolic sequelae, a better knowledge of the broad mode of action of this system will allow modulating it in a more selective and specific way, thus outweighing or reducing possible side effects associated with therapy.

Sources of Research Support: INSERM/Avenir grant; INSERM/ Interface; French Society of Endocrinology; European Federation for the Study of Diabetes; Fondation Recherche Medicale; Region Aquitaine; FP7-Marie Curie Reintegration Grant.

Nothing to Disclose: DC

Pub # S6-1

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Nutrient-Sensing Pathways in Longevity (9:30 AM - 11:00 AM)

Title Genetic Signature of Extreme Longevity

Author String P Sebastiani
Boston University, Boston, MA

Body Numerous studies suggest that most people have the genetic make-up to live to their mid to late eighties and therefore much of the variation of living to around these ages is explained by health related behaviors. On the contrary, exceptional longevity (EL) has a strong genetic predisposition that is possibly determined by different combinations of longevity enabling variants. We performed a genome wide association study (GWAS) of 801 centenarians (ages 100-119 yrs) and 926 population-matched controls drawn from a genome-wide data sample of approximately 4,000 subjects provided by Illumina. To determine replication of these findings, we performed a similar GWAS for 254 independently enrolled centenarians and 341 population-matched Illumina controls. We identified and replicated single nucleotide polymorphisms (SNPs) that met genome-wide significance using both frequentist and Bayesian analyses. While these individual SNPs are of interest, we conjectured that the combinations of alleles of these SNPs may contain added information about the genetic base and the different paths to EL. We therefore built a genetic model that includes more than 200 SNPs to predict EL with high accuracy in an independent set of centenarians and controls. We used this model for in-silico analysis of the genetic basis of EL and showed that 90% of centenarians in the NECS can be grouped into groups characterized by different combinations of SNP genotypes -or genetic signatures- that have varying degrees of predictive value. We were able to replicate these results and we also showed that these genetic signatures correlate with different life and health-spans. For example, the most predictive signatures are comprised of centenarians with the most extreme longevity and the latest ages of onset for dementia, hypertension and cardiovascular disease. Other genetic signatures characterize subtypes of centenarians with different ages of onset for specific age related diseases. Interestingly, centenarians do not appear to carry a smaller number of disease associated variants compared to controls, but rather they appear to be enriched of variants that protect against the effect of deleterious mutations.

Sources of Research Support: NIH/NHLBI Grant R01 HL68970.

Nothing to Disclose: PS

Pub #	S6-2
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Nutrient-Sensing Pathways in Longevity (9:30 AM - 11:00 AM)
Title	Calorie Restriction Delays Disease Onset & Mortality in Rhesus Monkeys
Author String	R Weindruch University of Wisconsin, Madison, WI
Body	Disclosure Incomplete: RW

Pub # S6-3

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Nutrient-Sensing Pathways in Longevity (9:30 AM - 11:00 AM)

Title Endocrine Approaches to Retarding Aging

Author String DE Harrison
The Jackson Laboratory, Bar Harbor, ME

Body The pituitary plays an important role in the regulation of mammalian aging. Hypophysectomy studies in mice and rats led to our analysis of lifespan in mice with specific hypophyseal endocrine deficiencies. We showed that the Snell dwarf mutation, which congenitally ablates growth hormone (GH), thyroid hormone and prolactin, increases lifespan by 25-50%. This mutation also reduces apparent rates of metabolic, collagen and immune aging, suggesting that it retards senescence. Bartke and colleagues made similar observations for the Ames dwarf mutation, which affects the same genetic pathway. Our studies of the [ldquo]little[rdquo] mutation, which inactivates the growth hormone releasing hormone receptor (Ghrhr) gene and virtually eliminates circulating GH and reduces circulating IGF1 by 90%, showed that GH deficiency alone increases lifespan by 20-25%. Kopchick, Bartke, and colleagues similarly showed that the GH receptor knockout mouse also lived 20-25% longer. All of these endocrine mutations also enhance insulin sensitivity and reduce circulating insulin. Thus, these endocrine deficiencies affect an evolutionarily conserved pathway -- the insulin/IGF1 signaling (IIS) transduction system -- to retard mammalian aging. To identify the molecular mechanisms, we are now evaluating specific components of the IIS pathway. As part of the NIA's Interventions Testing Program, we treated mice with rapamycin, an inhibitor of mTOR (mammalian target of rapamycin). mTOR mediates a downstream branch of the IIS pathway by integrating nutrient sensing signals with endocrine growth and maintenance signals. Rapamycin increased lifespan in the mice, indicating that this branch of the IIS pathway participates in the regulation of lifespan. Our own studies in BALB/cByJ mice showed that rapamycin diminished stem cell aging. Chen et al. (2009) showed that, in aging C57BL/6J mice, rapamycin improved response to flu vaccination. In a preliminary study of a different major branch of the IIS pathway, we evaluated the role of the transcription factor FOXO1 in aging. IIS activity inhibits FOXO1-dependent transcription. In mice transgenic for a mutant Foxo1 gene, which codes for a constitutively active FOXO1 protein, lifespan is increased. This result suggests that the IIS pathway influences aging through multiple branches. These studies demonstrate that the IIS pathway is an important regulator of aging and lifespan that can be altered through both genetic and pharmacologic means.

Co-Author: Kevin Flurkey, The Jackson Laboratory

Nothing to Disclose: DEH

Pub # S7-1

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Ovarian Follicular Development In Vitro: Implications for Fertility Preservation (9:30 AM - 11:00 AM)

Title "Waking Up" Dormant Ovarian Follicles

Author String AJW Hsueh
Stanford University School of Medicine, Stanford, CA

Body Although multiple follicles are present in mammalian ovaries, most of them remain dormant for years or decades. During reproductive life, only a small number of follicles are activated for development per month. Using an inhibitor of PTEN (Phosphatase with TENsin homology deleted in chromosome 10) and a PI3K (phosphatidylinositol-3-kinase) activating peptide mimicking the phosphorylated intracellular region of the activated PDGF receptor, we found that short-term treatment of neonatal mouse ovaries increased nuclear exclusion of Foxo3 in primordial oocytes. After transplantation under kidney capsules of ovariectomized hosts, treated follicles developed to the preovulatory stage with mature eggs displaying normal epigenetic changes of imprinted genes. After in vitro fertilization and embryo transfer, healthy progeny with proven fertility were delivered. In addition to the activation of dormant follicles from neonatal mice, a similar in vitro activation and allo-transplantation protocol also allowed the activation of residual follicles in aging mice at >one year of age to yield mature oocytes. Furthermore, we set up a two-step in vitro culture system and activated primordial follicles in ovarian explants obtained from neonatal mice, followed by further culturing with GDF9 for 11 more days to obtain preantral follicles. These preantral follicles containing an intact theca layer were then dissected out mechanically from activated ovaries and cultured on an ovarian stromal cell feeder layer. After another 10~12 days of culture on feeders, we obtained preovulatory follicles, a fraction of them respond to hCG and EGF to yield mature oocytes capable of undergoing germinal vesicle breakdown (GVBD). Because human primordial follicles in ovarian cortical fragments of patients with benign ovarian tumor could also be activated and xeno-transplanted into immune-deficient mice to yield mature oocytes, future studies could involve short-term in vitro activation of residual dormant ovarian follicles from patients with primary ovarian insufficiency (POI) or from peri-menopausal women with a diminishing ovarian reserve to allow auto-transplantation and the generation of mature oocytes. In addition, generation of a large number of human oocytes using the xeno-transplantation approach could facilitate future derivation of embryonic stem cells for regenerative medicine.

Sources of Research Support: NICHD grant R21 HD060864.

Nothing to Disclose: AJWH

Pub # S7-2

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Ovarian Follicular Development In Vitro: Implications for Fertility Preservation (9:30 AM - 11:00 AM)

Title Optimizing In-Vitro Maturation of Oocytes from Small Antral Follicles

Author String J Smitz
Universitair Ziekenhuis Brussel, Brussels, Belgium

Body Oocyte in vitro maturation (IVM) is an established technique in many animal species, but has yet to make a significant impact as a treatment option for infertile couples. IVM of human oocytes provides options in the treatment of infertility, especially for women with polycystic ovarian syndrome, for couples who cannot afford ovarian hyperstimulation and for couples where male factor infertility is the only identified cause of infertility.

IVM has been limited in particular by reduced embryo development and approximately 10% implantation rate (versus 20-30% in IVF) following embryo transfer, with high levels of early miscarriage (25% vs. 15% for IVF). There is no evidence that IVM is associated with perturbations in fetal and neonatal growth or congenital malformations above that seen within conventional IVF (approximately 5% of births). IVM reduces the risk of ovarian hyperstimulation syndrome (OHSS) and related complications as compared to conventional IVF. There are also notably reduced costs per cycle to couples and health care providers as less monitoring is needed.

In an unstimulated IVM cycle, oocyte pick-up is performed in a natural cycle when the lead follicle is ~10mm in diameter or in an anovulatory cycle when the endometrial thickness is at least 5mm. In these conditions, oocyte maturation occurs in vitro over a 30-36 h period and is referred to as spontaneous IVM. In some cases a brief FSH priming is used to increase oocyte numbers. Another protocol involves administration of hCG (10,000 I.U.), which appears to initiate a cascade of molecular events which possibly mimics an ovulatory cascade, perhaps inducing a blunted ovulatory EGF-like cascade in the small follicles.

Literature shows that altering laboratory conditions during IVM can significantly enhance not only blastocyst production yields, but also fetal survival following transfer: recent advances include the use of FF- cAMP modulating agents such as phosphodiesterase inhibitors and oocyte-secreted factors such as GDF-9 and BMP-15 into the culture medium. Appropriate metabolic conditions and the importance of gap-junctional and paracrine communication within the cumulus-oocyte complex during IVM is fundamental for the attainment of oocyte developmental competence.

The latest achievements from most recent clinical studies will be discussed.

Nothing to Disclose: JS

Pub # S7-3

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Ovarian Follicular Development In Vitro: Implications for Fertility Preservation (9:30 AM - 11:00 AM)

Title In Vitro Maturation of Ovarian Follicles

Author String TK Woodruff
Northwestern University, Chicago, IL

Body Three-dimensional in vitro follicle culture is an emerging technology that may be a viable fertility preservation option for patients who have cryopreserved ovarian tissue but are not eligible for ovarian transplant. In this in vitro culture system, immature ovarian follicles are grown in an alginate hydrogel that supports follicular architecture through the antral stages. Murine follicles, grown from the two layer secondary stage with this method, produce fertilization-competent female gametes capable of giving rise to live offspring. The development of competent human and non-human primate follicles requires extended culture periods and a bioengineered environment that mimics the physical structure of the ovary. Moreover, stroma cells and integrin cues are critical to the development of high quality oocytes. By engineering the in vitro environment to mimic in vivo structure-function relationships, we hope to create an artificial ovary that can support routine maturation of follicles for fertility preservation as well as to learn more about the biology of the growing follicle.

Nothing to Disclose: TKW

Pub # S8-1

Session Information SYMPOSIUM SESSION: CLINICAL - Aromatase Inhibitors in Pediatrics (9:30 AM - 11:00 AM)

Title Aromatase Inhibitors: The Good, Bad & Unknown

Author String M Hero
University of Helsinki/Central Hospital, Helsinki, Finland

Body Aromatase inhibitors (AIs), blockers of estrogen biosynthesis, have emerged as a new potential option for the treatment of children with poor adult height prognosis. Recent randomized placebo-controlled studies with third-generation AIs, letrozole and anastrozole, have shown that these compounds effectively delay bone maturation and increase predicted adult height at least in boys with idiopathic short stature, constitutional delay of growth and puberty, and growth hormone deficiency. The treatment effect on final adult height, however, is still not well characterized.

The safety of AI treatment in growth indications has received attention during recent years. Treatment of pubertal males with potent AIs result in stimulated gonadotropin and testosterone secretion and enhanced testicular growth and pubertal maturation. Serum estradiol concentrations remain at the prepubertal level. The pubertal increase in IGF-I is blunted, probably due to suppression of growth hormone secretion. As evaluated by DXA, treatment with AI does not interfere with normal accrual of bone mass during adolescence in males. However, slight differences in markers of bone turnover have been found between adolescent boys treated either with the AI letrozole or placebo, suggesting that letrozole lowers the rate of bone turnover. Whereas the effects of AI-therapy specifically on trabecular bone has not been investigated in humans, cortical bone growth appears be stimulated by the treatment-induced increase in testosterone-to-estradiol ratio. On the other hand, treatment with letrozole during prepuberty or early puberty may interfere with normal vertebral body growth.

In pubertal boys, AI-treatment appears to influence some risk factors for cardiovascular disease. Stimulated testosterone secretion decreases the percentage of fat mass and the level of HDL-cholesterol, while LDL-cholesterol and triglycerides remain unchanged. Hemoglobin level may also increase. Limited available evidence suggests that maturing spermatogenesis and cognitive performance is not influenced. Future studies should clarify the optimal skeletal maturity at treatment onset and the optimal treatment duration. Further, future prospective studies should clarify the impact of AIs on BMD and bone quality in different compartments of bone, and on vertebral body growth. Other safety issues to be addressed include treatment effect on maturing spermatogenesis, vascular wall function and prostate growth.

Nothing to Disclose: MH

Pub # S8-2

Session Information SYMPOSIUM SESSION: CLINICAL - Aromatase Inhibitors in Pediatrics (9:30 AM - 11:00 AM)

Title Aromatase Inhibitors: Therapeutic Uses in Males

Author String JM Wit
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Body The availability of the 3rd-generation Aromatase Inhibitors (AIs) (anastrozole 1 mg, letrozole 2.5 mg, exemestane 25 mg) and clinical observations in males with aromatase deficiency have stimulated the therapeutic use of AIs in male adolescents for 3 purposes: 1) to suppress pubertal gynecomastia; 2) as an adjunct to antiandrogens in gonadotrophin-independent precocious puberty (familial male-limited precocious puberty (FMPP) and McCune-Albright syndrome) and congenital adrenal hyperplasia (CAH), in addition to GnRH-analogues when central precocious puberty becomes evident; and 3) to increase adult height in short boys with or without pubertal delay.

1. Gynecomastia: In a randomized controlled study no effect of anastrozole was observed after 6 months, but recent open-label study suggested some effect.

2. Precocious puberty: In FMPP, positive results have been published about the combination of anastrozole and bicalutamide (a potent antiandrogen), and a phase II study is currently underway. Interim results of a long-term clinical trial in CAH evaluating the addition of the AI testolactone (later switched to letrozole) and the antiandrogen flutamide to low-dose hydrocortisone and fludrocortisone are promising.

3. Short stature: Four randomized studies have been published on the effect of AIs in short adolescent boys. In a Finnish study the combination of 1 year of letrozole with 6 months testosterone (T) in boys with delayed puberty led to a 5.1 cm increase in predicted adult height (PAH) 18 months after the start of the study, and adult height was 6.9 cm taller than with T alone. In a 2nd Finnish study, (mostly prepubertal) boys with idiopathic short stature (ISS) were treated for 2 years with letrozole. They showed an increase of predicted adult height (PAH) of 5.9 cm after 2 years, but 4 years later this had decreased to 4 cm (NS). Vertebral deformities were found in 5/11 patients. A 3rd study (from the US) assessing the additional effect of 3 years anastrozole treatment in growth hormone (GH) treated pubertal boys showed a PAH gain of 6.7 versus 1 cm in controls, but no adult height data are available yet. A 4th study (performed in Iran) assessed the effect of 2 years letrozole in 91 short boys, in comparison to placebo or oxandrolone. Letrozole increased PAH by 6.1 cm in comparison to 1.4 and 1.9 cm in both other arms.

In conclusion, for all these indications the use of AIs is experimental, and the safety profile is still insufficiently known.

Nothing to Disclose: JMW

Pub # S8-3

Session Information SYMPOSIUM SESSION: CLINICAL - Aromatase Inhibitors in Pediatrics (9:30 AM - 11:00 AM)

Title Aromatase Inhibitors in McCune-Albright Syndrome

Author String SB Nunez
Specially for Children, Austin, TX

Body McCune-Albright Syndrome (MAS) is a rare, sporadic condition consisting of the classic triad of gonadotropin-independent precocious puberty (GIPP), polyostotic fibrous dysplasia, and café-au-lait pigmentation. This is due to postzygotic activating missense mutation (Cys or His to Arg²⁰¹) in the G_{sα} gene (1) resulting in the constitutive activation of adenylyl cyclase -dependent receptors. It commonly manifests with sudden onset of vaginal bleeding ±breast development due to dysregulated estrogen production from large ovarian cysts independent of LH and FSH. The estrogen stimulation also causes accelerated growth, rapid bone age maturation leading to premature epiphyseal fusion resulting in shorter adult stature. Ideal therapy would involve an estrogen antagonist such as tamoxifen or an aromatase inhibitor (AI) that blocks estrogen synthesis however, these therapies have been challenging. Data from a multicenter clinical trial using tamoxifen for 12 months (2) reported a reduction in vaginal bleeding, rates of linear growth, and bone age advancement (BA/CA). Long-term data regarding the safety and efficacy of tamoxifen in MAS is not available. Early studies with an AI, testolactone was partially effective but puberty resumed after 2-4 years of treatment (3). Our studies using a nonsteroidal AI, fadrozole, was ineffective in a group of girls even when combined with a GnRH agonist (4).
A more potent AI, letrozole became available for the treatment of estrogen-dependent breast cancer. Data on letrozole indicates 95% suppression of estrogen level in treated women. So, letrozole was used in a clinical trial given continuously up to 48 mos in girls with MAS. The data after 12, 24 and 36 mos of treatment showed decrease in growth velocity SDS and BA/CA changes. Vaginal bleeding ceased in more than half of the girls while in some the frequency decreased. The pubertal stages of breasts and pubic hair stabilized. The mean serum estradiol and mean ovarian volumes (MOV) fell significantly after 6 mos of treatment but increased towards baseline levels by 12 and 24 mos. felt to be due to increase in ovarian cyst volumes (5). These data indicate that letrozole can be an effective initial therapy for GIPP and may be used as an alternative in girls who failed to respond to tamoxifen. More studies are needed to establish the safety and long-term efficacy of aromatase inhibitors in GIPP associated with MAS.

- (1) Weinstein LS et al., N Engl J Med 1991;325:1688
- (2) Eugster EA et al., J Pediatr 2003;143:60-6
- (3) Feuillan PP et al., J Clin Endocrinol Metab 1998;77:647
- (4) Nunez SB et., J CLin Endocrinol Metab 2003;88:5730
- (5)Feuillan P et al., J CLin Endocrinol Metab 2007;92:2100

Nothing to Disclose: SBN

Pub # S9-1

Session Information SYMPOSIUM SESSION: CLINICAL - Autoimmune Thyroid Disease: What's New in Clinical Practice? (9:30 AM - 11:00 AM)

Title Controversies in the Management of Graves Disease in Children

Author String CA Dinauer
Yale University School of Medicine, New Haven, CT

Body Graves' Disease (GD) is the most common etiology of hyperthyroidism in children. Establishing the diagnosis is relatively straightforward, but controversy surrounds the management. Treatment options include medication, surgery and radioactive iodine (RAI). In children, medical therapy is usually the initial treatment; in the U.S., two antithyroid drugs have been the mainstays: propylthiouracil (PTU) and methimazole (MMI). However, in 2007, an increased incidence of liver failure and deaths was recognized in children receiving PTU compared with adults (1). Hearings led to the recommendation that PTU never be used as first-line treatment of GD in children (2,3). Professional medical societies, including The Endocrine Society, issued statements supporting this recommendation (4). Despite this, some practitioners continued to prescribe PTU and additional cases of liver failure in children were reported. The U.S. Food & Drug Administration subsequently issued a safety alert in 2009, followed by a boxed warning in April 2010. Since MMI is effectively now the only available antithyroid drug for children, patients who experience side effects must be referred for definitive therapy earlier in their Graves' disease course. For those patients who tolerate MMI, there is debate about duration of treatment, given data showing no increased likelihood of remission after one year (5). The pros and cons of the two definitive treatment options, surgery and RAI, continue to be discussed. Clinical factors play a role in determining which route to take. Children who do not tolerate MMI and are acutely thyrotoxic, have very enlarged thyroid glands (>80 gm), or are prepubertal may be referred to surgery. Total thyroidectomy is considered the appropriate operation for GD, to minimize risk of recurrence (6). The risks of surgery, including temporary hypocalcemia, bleeding, recurrent laryngeal nerve injury, and permanent hypoparathyroidism, are not trivial. There is evidence that a surgeon's volume of thyroid cases is more important than their training path (7). RAI treatment is appealing in that it is non-invasive, less expensive than surgery, and one dose is curative 95% of the time (8). However, use in pediatrics is debated, with some expressing long term safety concerns for young children. Dosing strategies vary, with some institutions using fixed dosages while others base dosing on estimated thyroid size; regardless, the goal should be to achieve a hypothyroid state.

(1) Rivkees S, J Pediatr Endocrinol Metab 2007; 20(9):953

(2) Eunice Kennedy Shriver National Institute of Child Health and Human Development./ Hepatic Toxicity Following Treatment for Pediatric Graves' Disease Meeting: October 28,2008. Conference Proceeding. <http://bpca.nichd.nih.gov/outreach/index.cfm>

(3) Rivkees S et al., N Engl J Med 2009;360:1574

(4) Endocrine Insider, April 15,2009; Society Issues Statement on Use of PTU in Children

(5) Hamburger J, J Clin Endocrinol Metab 1985;60:1019

(6) Miccoli P, World Surg 1996;120(6):1020

(7) Sosa J, J Clin Endocrinol Metab 2008; 93(8):3058

(8) Rivkees S, Pediatrics 2003; 111(4 Pt 1);745

Nothing to Disclose: CAD

Pub # S9-2

Session Information SYMPOSIUM SESSION: CLINICAL - Autoimmune Thyroid Disease: What's New in Clinical Practice? (9:30 AM - 11:00 AM)

Title New Approaches to the Management of Graves Eye Disease

Author String RS Bahn
Mayo Clinic, Rochester, MN

Body The ideal approach to management of Graves' ophthalmopathy (GO) puts emphasis on limiting progression of existing disease. Once established, the extensive tissue remodeling seen in the GO orbit can be at best only imperfectly reversed or modulated through aggressive immunotherapy or surgical intervention. The choice of treatment modality for Graves' hyperthyroidism, and decisions concerning prophylactic corticosteroids when radioactive iodine is used, have bearing on disease outcome. In addition, the avoidance of hypothyroidism at any point in the course of the disease is an important clinical goal. To mitigate against the impact of the smoking, an active smoking cessation program should be recommended to all hyperthyroid patients and passive smoking avoided. Treatment of established GO requires a carefully integrated approach involving the endocrinologist and ophthalmologist, with the goal of preserving the patient's vision and restoring favorable self-perception and quality of life. Determining the appropriate treatment rests on assessing whether the eye disease is active or inactive, and defining the severity of the ocular manifestations. Most patients with GO will have a self-limited and mild disease course requiring only symptomatic relief and careful follow-up. Recent studies suggest that these patients may benefit from the anti-oxidant selenium selenite, 200 mg daily. Patients with moderate to severe and active GO may benefit from immunosuppressive therapy. There is good evidence that intravenous corticosteroids are more effective than oral in this setting. Orbital radiotherapy by itself, or in combination with corticosteroids, may also be of benefit to patients with active GO, especially those with extraocular muscle dysfunction. Patients with moderate to severe disease manifestations who show little disease activity are typically candidates for extraocular muscle surgery and/or orbital decompression, the latter also being appropriate in selected individuals with active disease. Compressive optic neuropathy may respond to high-dose IV corticosteroids; if ineffective after 2 weeks, orbital decompression surgery is indicated. The efficacy of rituximab, an anti-CD20 monoclonal antibody, in the treatment of active GO is unclear and currently being evaluated in 2 randomized controlled trials. Other novel agents, considered to be candidates based on disease pathogenesis, await similar trials.

Nothing to Disclose: RSB

Pub # S9-3

Session Information SYMPOSIUM SESSION: CLINICAL - Autoimmune Thyroid Disease: What's New in Clinical Practice? (9:30 AM - 11:00 AM)

Title "Elevated" Thyroid-Stimulating Hormone in the Elderly: Physiologic or Pathologic

Author String MI Surks
Montefiore Medical Center, Bronx, NY

Body **The Problem:** TSH in 10 to 15 % of the elderly (> 70 years of age) is raised above the accepted upper reference limit, 4.5 mIU/L; in 93 % it is only minimally raised, < 10 mIU/L. Do all have hypothyroidism? **Pathologic?** The prevalence of thyroid antibodies (AB) increases with age, up to 25 % in the elderly, but AB prevalence in those with raised TSH, 70 % in 50 year olds, decreases to 40 % in the elderly. The majority of elderly with raised TSH do not have AB. Biochemically, hypothyroidism depends on defining the TSH upper reference limit, derived from TSH distribution curves. The curve is traditionally determined from TSH in people of all ages and races/ethnicities. We reported that the distribution curve progressively shifts to higher TSH values with age; the upper limit of elderly people is 7.5 mIU/L based on age-specific TSH distribution curves. Of elderly currently classified hypothyroid, TSH > 4.5 mIU/L, 60 % have TSH within age-specific limit, suggesting that the prevalence of subclinical hypothyroidism may be significantly overestimated. Distribution curves and limits for blacks and Hispanic-Americans in the Bronx shift to lower TSH than in Caucasians, but the age shift to higher values occurred in all subpopulations. Thus, age- and race/ethnicity-specific limits need be employed to minimize misclassification of patients with thyroid disease. Using these demographic data, we present model equations that predict upper and lower TSH limits which can be programmed into laboratory computers.

Physiologic or Beneficial?
Animal models demonstrate an association between hypothyroidism and longevity. 85 year-old subjects with raised TSH survived longer than those with normal TSH, without deficits in activities of daily living or quality of life. TSH distribution of centenarians was shifted to higher values than their offspring, whose TSH, distribution, in turn, was shifted to higher values than their spouses (age-matched). A significant hereditary influence on TSH was noted between centenarians and their offspring, associated with 2 SNPs in the coding region of the TSH receptor. The prevalence of these SNPs was greatest in centenarians, declined in their offspring, and was lowest in spouses of offspring, suggesting a heritable phenotype of raised TSH and longevity. Additionally, longevity of parents was recently correlated with TSH in 90 year-old siblings. These recent findings suggest a potential benefit of minimally raised TSH in the elderly.

Hollowell JG, Boucai L, Atzmon G, Gabriely I, Barzilai N.

Nothing to Disclose: MIS

Pub # S10-1

Session Information SYMPOSIUM SESSION: CLINICAL - Consequences of Androgen Excess in Women (9:30 AM - 11:00 AM)

Title Cardiovascular Dysfunction in Polycystic Ovary Syndrome

Author String F Orio
University "Parthenope" Naples and University Hospital Salerno, Salerno, Italy

Body Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disease that occurs up to 10% of reproductive age women. PCOS is considered not only a reproductive endocrinopathy, but also a metabolic disorder and a multifaceted disease associated with long-term health risks, including diabetes mellitus and coronary artery disease. In particular, insulin resistance, hyperandrogenemia and dyslipidemia are likely the major risk factors for the occurrence of cardiovascular disease (CVD) in PCOS. These cardiovascular risk factors are often evident at an early age, suggesting that women with PCOS represent a large group of women at increased risk for developing early onset CVD. Because of its association with insulin resistance, PCOS can lead to several metabolic complications. Although prospective controlled data on CVD morbidity and mortality in PCOS patients are lacking, various risk factors for CVD are detected in the PCOS population. In particular, PCOS subjects have an increased incidence of obesity, hyperinsulinemia, hypertension, dyslipidemia, elevated plasminogen activator inhibitor-1 (PAI-1) and endothelin 1 (ET-1), increased serum advanced glycation end-products, a reduced cardiopulmonary functional capacity, endothelial disarrangements, chronic inflammation, and impaired fibrinolysis. The pathogenetic mechanisms accounting for these abnormalities are still controversial, but several evidences suggested a relationship with both hyperandrogenism and impaired peripheral insulin sensitivity.

One of the early signs of cardiovascular lesions is the endothelial injury. It was reported precocious anatomical and functional arterial changes in PCOS women.

The risk of coronary artery disease and myocardial infarction has been reported to be increased in patients with PCOS compared to regularly cycling women even if mortality because of circulatory disease does not seem to be increased. However, from a recent statement, PCOS women with metabolic syndrome and/or type 2 diabetes mellitus and overt vascular or renal disease are at high risk for CVD.

Lifestyle management is recommended for primary CVD prevention targeting low-density lipoprotein cholesterol and adding insulin-sensitizing drugs. In conclusion further additional controlled study are needed to clarify the true impact of PCOS on the cardiovascular dysfunction and in order to demonstrate the detrimental effect of PCOS on the cardiovascular system even in young women asymptomatic for cardiac disease.

Nothing to Disclose: FO

Pub # S10-2

Session Information SYMPOSIUM SESSION: CLINICAL - Consequences of Androgen Excess in Women (9:30 AM - 11:00 AM)

Title Hepatic Dysfunction in Polycystic Ovary Syndrome

Author String HF Escobar-Morreale
Hospital Universitario Ramon y Cajal, Madrid, Spain

Body Patients with polycystic ovary syndrome (PCOS) suffer a vicious circle consisting of androgen excess favoring abdominal visceral adiposity, insulin resistance, hyperinsulinism, and further androgen secretion by the adrenals and the ovaries (1). The liver plays a central role in the pathophysiology of PCOS, and in the development of the metabolic derangements associated with this syndrome. Although androgens are not generated by the liver, hepatic secretion of sex hormone-binding globulin (SHBG) is central for androgen circulation in blood and the liver is also responsible of the catabolism and inactivation of androgens. SHBG levels actually determine the amount of androgens that reach target tissues. SHBG secretion is negatively regulated by androgens and insulin, and stimulated by estrogens and thyroid hormones, but is also influenced by genetic variation. Accordingly, patients with PCOS characteristically present with decreased circulating SHBG levels, resulting from androgen excess and from insulin resistance and portal hyperinsulinism, and PCOS is associated with several variants in the SHBG gene that influence its circulating levels (2). Considering the central role that SHBG plays in PCOS, it is not surprising that decreased SHBG levels are possibly the most accurate individual marker of PCOS, with an area under the ROC curve of 0.88 (95% CI 0.80 - 0.93). A SHBG decision threshold of 37 nmol/l had a sensitivity of 88% and a specificity of 87% for the diagnosis of PCOS (3). Furthermore, increasing SHBG levels is one of the most important factors associated with the amelioration of hyperandrogenic symptoms in response to oral contraceptives in patients with PCOS. But liver dysfunction may also be the consequence of the metabolic milieu associated with PCOS. The association with abdominal obesity, insulin resistance and dyslipidemia contributes to explain why non-alcoholic fatty liver disease is a frequent finding in patients with PCOS, especially when abdominal obesity is also present (4). Finally, the liver is also the central organ for iron metabolism and storage. Body iron stores are increased in patients with PCOS because of decreased hepcidin levels (5) - hepcidin is the major limiting factor of intestinal iron absorption - and the increase in body iron stores is especially important in patients presenting with abnormal glucose tolerance, suggesting a pathogenetic link between iron excess and the development of diabetes in this population (6).

- (1) Escobar-Morreale HF, San Millán JL, Trends Endocrinol Metab 2007;18:266
- (2) Wickham et al., J Clin Endocrinol Metab doi:10.1210/jc.2010-1842
- (3) Escobar-Morreale HF et al., Eur J Endocrinol 2001;145:619
- (4) Baranova et al. Aliment Pharmacol Ther doi:10.1111/j.1365-2036.2011.04579.x
- (5) Luque-Ramírez et al., J Clin Endocrinol Metab doi:10.1210/jc.2010-2211
- (6) Escobar-Morreale HF et al., Diabetes Care 2005;28:2042

Sources of Research Support: Grant FIS PI 080944, ISCIII, MCINN; CIBER Diabetes y Enfermedades Metabólicas Asociadas CIBERDEM; Instituto Ramón y Cajal de Investigación Sanitaria IRYCIS.

Nothing to Disclose: HFE-M

Pub # S10-3

Session Information SYMPOSIUM SESSION: CLINICAL - Consequences of Androgen Excess in Women (9:30 AM - 11:00 AM)

Title Dyslipidemia in Polycystic Ovary Syndrome

Author String TL Setji
Duke University Medical Center, Durham, NC

Body Dyslipidemia occurs in up to 70% of women with polycystic ovary syndrome (PCOS) in the United States. The atherogenic lipoprotein profile (low high-density lipoprotein [HDL]-cholesterol, elevated triglyceride concentrations, and increased small, dense low-density lipoproteins [LDL]) is relatively common and is likely related to insulin resistance. Increased LDL-cholesterol has also been reported, but studies are conflicting on this and generally reveal milder abnormalities. The elevation in LDL-cholesterol appears to be related in part to hyperandrogenemia. In addition to insulin resistance and hyperandrogenemia contributing to dyslipidemia in PCOS, there are likely genetic and environmental factors contributing to the variability in patterns and severity of dyslipidemia. These differences are highlighted in studies comparing women with PCOS from the Mediterranean versus the United States. Additionally, within the United States, differences in lipid profiles are seen between African American and Caucasian women with PCOS. For screening, women with PCOS should have a fasting lipid profile performed. Measuring lipid subfractions has also been useful in research settings. First line treatment of dyslipidemia, particularly in young women, is lifestyle intervention including a hypocaloric diet low in saturated fat and regular physical activity. Even without weight loss, moderate-intensity exercise can improve dyslipidemia in PCOS. Pharmaceutical agents, such as statins, nicotinic acid, fenofibrate, or omega-3 fatty acids can be considered in more severe cases.

Nothing to Disclose: TLS

Pub #

Session Information

CONFERENCE EVENT: ENDO's Got Talent (11:00 AM - 12:30 PM)

Title

ENDO's Got Talent

Author String

Body

Pub # LB-1

Session Information ORAL SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (11:15 AM-12:45 PM)

Title Potent Anti-Diabetic Effects of a PPAR γ Ligand Devoid of Classical Agonism That Blocks Cdk5-Mediated Phosphorylation

Author String AS Banks, JH Choi, TM Kamenecka, MJ Chalmers, PR Griffin, BM Spiegelman
Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA; The Scripps Research Institute, Jupiter, FL

Body PPAR γ is the target of highly effective but controversial diabetes medications, the thiazolidinediones (TZDs). We recently demonstrated that these drugs reverse insulin resistance through a previously unappreciated mechanism--reversing an inhibitory PPAR γ phosphorylation event. With progressive obesity in mice, we find activation of Cdk5 protein kinase and phosphorylation of PPAR γ at serine 273 in adipose tissue. This modification does not affect the adipogenic capacity of PPAR γ , but leads to the dysregulation of a large number of target genes including the insulin sensitizing adipokine, adiponectin. TZDs are strong PPAR γ agonists, yet we find that even weak agonists have potent glucose-lowering properties. We therefore postulated that agonist activity may be dispensable for the anti-diabetic effects of PPAR γ ligands. Here we demonstrate that a non-agonist PPAR γ modulator (NAPM) which strongly blocks CDK5-mediated phosphorylation is the first of a new class of therapeutic diabetes agents. Our novel NAPM does not promote transcriptional activity on the classical DR1 PPAR responsive element. This compound also does not enhance adipocyte differentiation or lipid accumulation in cultured cells. However it is strongly blocks CDK5-mediated PPAR γ phosphorylation both in vitro and in cells. Mice treated with either a NAPM or TZD have dramatic improvements in glucose tolerance, yet unlike with TZD treated mice, animals administered NAPM do not develop increased fluid retention or gain excess body weight. Although further preclinical testing is necessary, the ability to retain the beneficial properties of the best PPAR γ ligands without the harmful side effects suggests a new therapeutic possibility for the treatment of type 2 diabetes.

Nothing to Disclose: ASB, JHC, TMK, MJC, PRG, BMS

Pub # LB-2

Session Information ORAL SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (11:15 AM-12:45 PM)

Title Heart-Gut Cross-Talk: BNP Modulates Ghrelin, Hunger and Satiety in Humans

Author String G Vila, G Grimm, M Resl, B Heinisch, A Luger, M Clodi
Medical University of Vienna, Vienna, Austria; Medical University of Vienna, Vienna, Austria; Medical University of Vienna, Vienna, Austria

Body Abstract Embargoed.

Sources of Research Support: A Research Grant of the Austrian National Bank to MC.

Nothing to Disclose: GV, GG, MR, BH, AL, MC

Pub # LB-3
Session Information ORAL SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (11:15 AM-12:45 PM)
Title Human Recombinant Leptin (Metreleptin) Administration for 2 Years Is an Effective Treatment for Hypothalamic Amenorrhea and Increases Bone Mineral Density at the Lumbar Spine: A Pilot Study
Author String KN Aronis, E Sienkiewicz, SH Chou, M Brinkoetter, JP Chamberland, KM Aramopatzis, C Gao, F Magos, CS Mantzoros
Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Harvard School of Public Health, Boston, MA; Beth Israel Deaconess Medical Center, Boston, MA

Body
Introduction: Hypothalamic amenorrhea (HA) due to strenuous exercise or chronic energy deficiency is associated with dysfunction of hypothalamic-pituitary-peripheral axes resulting in infertility, bone loss and stress fractures. We have previously shown that HA is associated with hypoleptinemia and that open label metreleptin administration in replacement doses for up to 12 weeks restores neuroendocrine and reproductive function and changes the levels of circulating bone markers. No prior study has described the effect of metreleptin administration on these outcomes in the context of a double blinded, randomized, placebo controlled trial (RCT) or has evaluated metreleptin's effects on bone mineral density (BMD) and bone mineral content (BMC).
Methods: We recently completed an RCT of metreleptin administration for 9 months, followed by a 3-month washout period and a 12-month open label extension. We randomized 20 women with exercise induced HA and circulating leptin levels < 5ng/ml to either metreleptin (initial dose: 0.08mg/kg/day) or placebo for 9 months; 6 subjects proceeded to receive open label metreleptin for an additional 12 months after the washout period. BMD and BMC of the total body, lumbar spine (L1-L4), hip and radius were assessed using DEXA at baseline and at 3, 6, 9, 12, 18, and 24 months of treatment. Metabolic, hormonal and bone parameters were measured in blood and urine at those time points.
Results: Metreleptin restored menstruation in ~75% of subjects and significantly increased BMD (P=0.02) and BMC (P=0.01) at the lumbar spine by 4-6% after two years of treatment. Metreleptin significantly altered several hormonal, metabolic and bone parameters during the first 9 months of treatment, and also increased circulating levels of IGF-1 and decreased cortisol and cross-linked C-terminal telopeptide of type I collagen during the second year of treatment in the context of the open label extension (all P-values <0.05).
Conclusions: Long-term metreleptin therapy in females with exercise-induced HA and hypoleptinemia improves not only neuroendocrine and reproductive function but also lumbar BMD and BMC and shifts bone formation/resorption balance towards bone formation. This pilot study should be extended by larger clinical trials that also study bone microarchitecture and fracture risk.

Sources of Research Support: National Institute of Diabetes and Digestive and Kidney Diseases grants 58785, 79929 and 81913, and AG032030. Funding was also received from the National Institutes of Health - National Center for Research Resources grant M01-RR-01032 (Harvard Clinical and Translational Science Center). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. Amylin Pharmaceuticals, Inc supplied recombinant human leptin for this study and approved the design of the study, but had no role in study design; conduct of the study; collection, management, analysis, and interpretation of the data; or the preparation, review, or approval of the manuscript.

Nothing to Disclose: KNA, ES, SHC, MB, JPC, KMA, CG, FM, CSM

Pub # LB-4

Session Information ORAL SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (11:15 AM-12:45 PM)

Title Meeting Men's Contraceptive Needs - Long-Term Oral-Administered Retinoic Acid Receptor Antagonist Inhibits Spermatogenesis in Mice with a Reversible and Rapid Recovery

Author String SSW Chung, X Wang, DJ Wolgemuth
Columbia University Medical Center, New York, NY

Body It has been recognized for decades that dietary retinol (vitamin A) and its physiologically active metabolite *all-trans*-retinoic acid are essential for normal spermatogenesis¹⁻⁴. Given the importance of retinoic acid receptor alpha (RAR α) in particular in regulating spermatogenesis⁵⁻⁸, we have investigated a pharmacological approach to inhibit spermatogenesis in the mouse model by manipulating retinoid signaling using low doses of pan-RAR antagonist BMS-189453. We have recently demonstrated that treatment of pan-RAR antagonist BMS-189453/compound 9 at 2.5 mg/kg/day for 4 weeks reversibly inhibited spermatogenesis in all drug-treated mice (Chung et al., Endocrinology, in press). There were no detectable abnormalities or adverse side effects except the distinct testicular pathology. To begin to further understand the lowest optimal dosing regimen and the longest period of inhibition of spermatogenesis after which fertility can still be restored, dosing regimens with extended dosing periods of 8 and 16 weeks and at an even lower dose of 1mg/kg/day were performed and the effects on spermatogenesis were compared to the 4-week dosing. Spermatogenesis was disrupted in all drug-treated mice, with morphology resembled those seen in the shorter dosing periods. Strikingly, our mating studies and morphological analyses suggested a more rapid recovery from the induced infertility in mice treated with extended dosing periods (16 weeks) as compared to 4 and 8 weeks of dosing. Sperm did not align properly at the lumen nor released. Instead, there was a loss of other germ cell types into the lumen. These abnormalities resembled those in vitamin A-deficient and RAR α -knockout testes⁵⁻⁸. Importantly, the sterility induced for longer periods remained reversible. There was no change in testosterone levels in the males treated with 16-week dosing which were terminated at various post-treatment time-points, suggesting long-term administration would not affect male libido or sexuality. To ensure that there were no adverse effects on progeny and their gametes, the resulting progeny of two recovered males after the 16-week dosing were allowed to mature. These progeny were healthy with normal fertility; males exhibited normal testicular weight and spermatogenesis. Our results suggest that testes are exquisitely sensitive to disruption of retinoid signaling and that RAR antagonists may represent new lead molecules in developing non-steroidal male contraceptives.

- (1) Howell JM et al., J Reprod Fertil 1963; 5:159
- (2) Wolbach SB and Howe PR, J Exp Med 1925; 42:753
- (3) Chung SSW and Wolgemuth DJ, Cytogenet Genome Res 2004; 105:189
- (4) Wolgemuth DJ and Chung SSW, Soc Reprod Fertil Suppl 2007; 63:11
- (5) Lufkin T et al., Proc Natl Acad Sci U S A 1993; 90:7225
- (6) Chung SSW et al., Dev Dyn 2004; 230:754
- (7) Chung SSW et al., Differentiation 2005; 73:188
- (8) Chung SSW et al., Development 2009; 136:2091

Sources of Research Support: NIH Grant U01-HD060479 awarded to DJW and SSWC.

Nothing to Disclose: SSWC, XW, DJW

Pub # LB-5

Session Information ORAL SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (11:15 AM-12:45 PM)

Title Long-Term Mortality after Recombinant Growth Hormone Treatment for Isolated Childhood Short Stature: Report of the French SAGhE Study

Author String J-C Carel, E Ecosse, F Landier, D Meguellati-Hakkas, F Kaguelidou, G Rey, J Coste
AP-HP Robert Debré Hospital and University Paris 7 Denis Diderot, Paris, France; Groupe Hospitalier Cochin - Saint Vincent de Paul and APEMAC EA 4360, University Paris Descartes, Paris, France; AP-HP Robert Debré Hospital, INSERM CIC9202, Paris, France; Inserm, Le Vésinet, France

Body Recombinant growth hormone treatment is widely used to promote growth in children with short stature. Little is known about the long-term safety of such treatment. SAGhE (safety and appropriateness of growth hormone treatments in Europe) is a large study evaluating the long-term health of individuals treated with recombinant growth hormone in childhood in eight European countries. We report on the mortality results of the study performed in France where it was initiated before the rest of Europe. This population-based cohort study was based on the French population-based register of children treated with growth hormone. 6928 short children diagnosed with idiopathic isolated growth hormone deficiency, idiopathic short stature or born short for gestational age who started recombinant growth hormone treatment between 1985 and 1996. Follow-up data on vital status were available in September 2009 for 94.7% of the patients, providing 116,403 person-years of observation. We measured all-cause and cause-specific mortality and calculated standardised mortality ratios (SMR) and hazard ratios (HR, using the category with the lowest exposure level as the reference). All-cause mortality was higher in treated children than in the general population (SMR 1.33, 95% CI 1.08-1.64). In multivariate analysis adjusted for height at the start of treatment, the use of high doses typically exceeding 50 [micro]g/kg/day was consistently associated with mortality rates almost three times those for the low-dose regimen, using both external and internal references (adjusted SMR 2.94, 95% CI 1.22-7.07, adjusted HR 2.79, 95% CI 1.14-6.82). Cancer-related mortality was not higher than for the general population, although bone tumour-related mortality was five times higher (SMR 5.00, 95% CI 1.01-14.63). An increase in mortality due to diseases of the circulatory system (SMR 3.07, 95% CI 1.40-5.83), and subarachnoid or intracerebral haemorrhage in particular (SMR 6.66, 95% CI 1.79-17.05), was also observed. In conclusion, mortality rates were significantly increased in this population of otherwise healthy but short children treated with recombinant growth hormone, particularly in those who had received the highest doses. Specific effects were detected in terms of death due to bone tumours or cerebral haemorrhage, but not for all cancers. These results highlight the need for additional studies of long-term mortality and morbidity after growth hormone treatment in childhood.

Sources of Research Support: The study was funded by Agence Francaise de Securite Sanitaire des Produits de Sante (AFSSAPS, the French drug safety agency), Direction Generale de la Sante (DGS, French Ministry of Health), Institut National du Cancer (INCa) and a Commission of European Communities grant (HEALTH F2-2009-223497).

J-CC: Investigator, Lilly USA, LLC; Scientific Board Member, Pfizer Global R&D. Nothing to Disclose: EE, FL, DM-H, FK, GR, JC

Pub # LB-6

Session Information ORAL SESSION: BASIC/TRANSLATIONAL/CLINICAL - Late Breaking Research in Endocrinology (11:15 AM-12:45 PM)

Title The Adrenal Vein Sampling International Study (AVIS) on Use and Interpretation of Adrenal Vein Sampling for Identifying the Major Subtypes of Primary Aldosteronism: Results of First Phase

Author String GP Rossi, M Barisa, B Allolio, R Auchus, G Kline, A Lacroix, JWM Lenders, SB Magill, M Naruse, T Nishikawa, PF Plouin, M Reincke, LC Rump, F Satoh, M Stowasser, CJ Strasburger, A Tanabe, S Trerotola, Widimsky, Jr, K-D Wu
University of Padova, Padova, Italy; University Hospital Würzburg, Würzburg, Germany; University of Texas, Dallas, TX; University of Calgary, Calgary, Canada; Université de Montréal (CHUM), Montreal, Canada; Radboud University, Nijmegen, Netherlands; Medical College of Wisconsin, Menomonee Falls, WI; National Hospital Organization, Kyoto, Japan; Yokohama Rosai Hospital, Yokohama City, Japan; Hospital Européen Georges Pompidou Paris, Paris, France; Medizinische Klinik Innenstadt, Munich, Germany; Heinrich Heine University Duesseldorf, Duesseldorf, Germany; Tohoku University Hospital, Sendai, Japan; University of Queensland School of Medicine, Greenslopes Hospital, Brisbane, Queensland, Australia; Charité Campus Mitte, Berlin, Germany; Institute of Clinical Endocrinology, Tokyo Women's Medical University, Tokyo, Japan; Hospital of The University of Pennsylvania, Philadelphia, PA; Center for Hypertension, General Faculty Hospital, Prague, Czech Republic; National Taiwan University Hospital, Taipei, Taiwan

Body **Objective.** The identification of the patients with primary aldosteronism (PA) who can be surgically cured requires differentiation between lateralised and non-lateralised causes of aldosterone excess. This can be achieved with adrenal venous sampling (AVS), which remains unfortunately a challenging task because of the possibility of adrenal vein rupture and of the lack of accepted criteria for use and interpretation of this test. We therefore performed a large international multicenter study to determine the complication rate of AVS and how this test is being performed and interpreted at major referral centers worldwide.

Design and method. The centers were identified through database search and invited to participate to the AVIS Study, which entails two phases: in the first summary data were collected, the second will provide individual patients data.

Results. The first phase ended in November 2010 and furnished data on rate of adrenal vein rupture and on percent of use of AVS in PA patients, modalities of performance and interpretation of the test. Of the 23 invited centers scattered all over Asia, Europe, North America, and Australia, 82.6% agreed to participate and furnished data on a total of 2595 AVS studies. The rate of adrenal vein rupture overall was extremely low (0.57%), but showed a significant heterogeneity across centers. A weak but significant correlation between complication rate and number of radiologists performing AVS at each center was found ($r=0.543$; $p<0.05$). The percentage of PA patients systematically submitted to AVS ranged from 40% to 100% (median 77.5). The sequential and the bilateral simultaneous catheterization technique were used at 63.2% and 36.8% of centers, respectively. ACTH stimulation was performed at 52.6% of the centers. A wide variability of cut-off values for the selectivity, lateralization, and contralateral suppression index existed. Some centers still used absolute hormonal values, instead of these indexes, for diagnosis.

Conclusions. At major referral centers worldwide AVS: 1) is being performed at a very low complication rate but is used in a highly variable proportion of the patients to be selected for adrenalectomy; 2) AVS is undertaken at most centers during ACTH stimulation even though this stimulation was shown to confound the assessment of lateralization; 3) notwithstanding the lack of any theoretical basis some centers continue to use absolute hormonal values instead of the appropriate indexes.

Nothing to Disclose: GPR, MB, BA, RA, GK, AL, JWML, SBM, MN, TN, PFP, MR, LCR, FS, MS, AT, ST, JW, K-DW

Pub # OR01-1

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (11:15 AM-12:45 PM)

Title Free Fatty Acids Inhibit β -Cell Proliferation by Inducing p16 and p18

Author String LC Alonso, DA Hollern, JL Pascoe, T Rosa, M Abbasi, LC Romano, B Zou, CP O'Donnell, A Garcia-Ocana
University of Pittsburgh, Pittsburgh, PA; University of Pittsburgh, Pittsburgh, PA

Body Beta cell mass is reduced in T2DM, and unbiased genome-wide association studies link diabetes risk with cell cycle regulatory genes, including the p16 locus. In obesity, elevated free fatty acids (FFA) are independently associated with progression to T2DM. To test whether FFA restrict beta cell proliferation in vivo, C57BL/6j adult male mice were infused intravenously with saline (SAL), Liposyn II (LIP), 50% dextrose (GLU), or Liposyn II with 50% dextrose (L+G), all with heparin (2U/hr) and bromodeoxyuridine (BrdU; 10mg/hr), for 4 days. GLU and L+G infusions increased circulating BG and plasma insulin ($p < 0.001$). LIP and L+G infusions increased plasma FFA ($p < 0.01$). As previously shown, glucose infusion (GLU) increased beta cell proliferation ($p < 0.01$ vs. SAL). Addition of Liposyn II (L+G) completely blocked glucose-induced beta cell proliferation ($p < 0.01$ vs. GLU, $p = ns$ vs. SAL). To determine whether the anti-proliferative effect of lipid was due to direct action of FFA on the beta cell, primary mouse islet cells were cultured in the presence of low (2mM) or high (15mM) glucose, with BSA control or a FFA mixture containing 7:2:1 linoleic [18:2]: oleic [18:1]: palmitic [16:0] acids (similar to Liposyn II FA components). Glucose-induced beta cell proliferation ($p < 0.01$ for 2mM+BSA vs. 15mM+BSA) was blocked by addition of FFA ($p < 0.01$ for 15mM+BSA vs. 15mM+FFA). In INS-1 cells, glucose-induced proliferation ($p < 0.01$ for 2mM+BSA vs. 11mM+BSA) was also blocked by FFA ($p < 0.01$ for 11mM+BSA vs. 11mM+FFA). In INS-1 cells the anti-proliferative effect of FFA was mediated by linoleic and palmitic acids. We hypothesized that FFA might prevent glucose-induced cyclin D2 protein stability or nuclear localization. However, in vivo, co-infusing lipid (L+G) altered neither the glucose-mediated increase in islet cyclin D2 protein nor its nuclear localization. In contrast, immunoblot showed that two cell cycle inhibitors downstream of D-cyclins, p16 and p18, were induced in L+G islets ($p < 0.01$ for L+G vs. GLU for p16; $p < 0.05$ for L+G vs. GLU for p18). In INS-1 cells, palmitic acid also induced p16 and p18 proteins ($p < 0.01$ for p16; $p < 0.001$ for p18). Remarkably, siRNA knockdown of either p16 or p18 rescued the anti-proliferative effects of palmitic acid in INS-1 cells. We conclude that FFA block glucose-induced beta cell proliferation by inducing p16 and p18, and we speculate that FFA may contribute to failure of beta cell mass expansion in T2DM.

Sources of Research Support: NIH DK076562 and an ONCR pilot and feasibility award DK046204 (LCA); HL063767 (COD); and DK067351 and DK077096 (AGO).

Nothing to Disclose: LCA, DAH, JLP, TR, MA, LCR, BZ, CPO, AG-O

Pub # OR01-2

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (11:15 AM-12:45 PM)

Title Parathyroid Hormone-Related Protein (PTHrP) 1-36 Markedly Enhances β -Cell Regeneration and Completely Restores β -Cell Mass in a Mouse Model of Partial Pancreatectomy

Author String A Mozar, H Lin, K Williams, N Guthalu, A Otero, C Chin, AF Stewart, AG Ocana, RC Vasavada
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Body A major focus in the field of diabetes is to identify molecules that enhance endogenous β -cell regeneration. Parathyroid hormone-related protein (PTHrP) holds great promise in this regard. PTHrP enhances rodent β -cell function, proliferation, and survival *in vitro*, and *in vivo* in PTHrP-overexpressing transgenic mice. The amino-terminal 1-36 peptide is sufficient to produce these beneficial effects *in vitro*, in rodent and human β -cells. Current use of PTHrP(1-36) in clinical trials for the treatment of osteoporosis attests to the safety of this peptide for clinical use. This study is the first to examine the therapeutic regenerative potential of PTHrP(1-36) in a partial pancreatectomy (PPx) mouse model of β -cell injury.

We have previously found that subcutaneous administration of PTHrP(1-36) at 80 or 160[μ g/kg] markedly enhances β -cell proliferation and mass by 25 days in eight-week old normal male BalbC mice. To determine its effect on β -cell regeneration after injury, we injected PTHrP(1-36) (160[μ g/kg] or vehicle (veh) in partially (50%) pancreatectomized (PPx) or sham-operated BalbC male mice (n=5-6 mice/group). There were no changes in body weight, blood glucose, insulin levels, insulin sensitivity or incidence of hypercalcemia in the four groups of mice. As expected, PPx mice were glucose intolerant at 1 month compared to sham-operated mice.

Importantly, at 1 week, PTHrP(1-36) significantly enhanced β -cell proliferation by 40% in PPx-PTHrP mice compared to the PPx-veh group. This increase in β -cell proliferation translated to a significant marked increase in β -cell mass at 3 months in the PPx-PTHrP (2.4 \pm 0.3 mg) versus PPx-veh mice (1.4 \pm 0.2 mg), causing a complete recovery of their β -cell mass, equivalent to the control sham-veh mice (2.6 \pm 0.3 mg). Interestingly, analysis of protein expression in islets from the four groups of mice after 1 week of treatment showed that PPx induces an increase in PTHrP receptor expression which is significant in the PPx-PTHrP group. Preliminary study of the cell-cycle proteins in islets from these treated mice suggests an increase in cyclin D2 and a decrease in p27 in PPx-PTHrP mice compared with PPx-veh mice.

Our results clearly demonstrate the regenerative potential of PTHrP(1-36) to restore endogenous depleted β -cell mass without negatively impacting glucose homeostasis. These studies together with its effects on human β -cells makes PTHrP(1-36) a strong candidate for the future therapeutic treatment of diabetes.

Sources of Research Support: Grants from the National Institutes of Health (DK078060 and DK072264) and JDRF Research Awards (1-2008-46).

Disclosures: AFS: Member, Osteotrophin LLC. Nothing to Disclose: AM, HL, KW, NG, AO, CC, AGO, RCV

Pub # OR01-3

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (11:15 AM-12:45 PM)

Title High-Fat Diet during Early Development Elicits β -Cell Expansion and an Increase in the β -Cell/ α -Cell Ratio in the Juvenile Nonhuman Primate

Author String SM Comstock, JM Bishop, DL Takahashi, AN Cornea, MS Smith, KL Grove
Department of Neuroscience, Beaverton, OR

Body Poor maternal health during pregnancy, including obesity and diabetes, causes both acute and long-term health issues in offspring. This includes increasing the risk for early onset obesity, diabetes and cardiovascular disease. The purpose of this study was to investigate the impact of early exposure to High Fat Diet (HFD) on the development of pancreatic islet cells and to determine if control (CTR) dietary intervention would lead to normalization of these effects.

Adult female Japanese macaques were maintained on either a CTR diet - 13% calories from fat or HFD - 35% calories from fat for 2 to 4 years. Fetal offspring were collected by c-section at 130 days of a 170 day gestation period. While islet mass was not significantly affected at this stage, fetal HFD offspring had decreased insulin and glucagon gene transcription. Genes involved in islet cell neogenesis and differentiation PDX1, NKX6.1 and NeuroD were also suppressed in HFD fetal pancreas.

After birth, infant animals were maintained on the same diet until weaning then either the CTR or HFD diet for 5 months. Animals were necropsied at approximately 14 months of age leading to four postnatal offspring groups CTR/CTR, CTR/HFD, HFD/CTR or HFD/HFD.

Body weights did not differ between juvenile offspring groups, but both HFD groups did have increased adiposity. Insulin secretion was significantly elevated in the HFD/HFD group while the CTR/HFD and the HFD/CTR group both displayed mild insulin resistance. The glucose transporter 2 was elevated in both HFD groups while glucokinase and insulin gene expression, was increased significantly in only the HFD/HFD group. This suggests altered mechanisms of glucose processing between the two HFD groups.

Islet characterization demonstrated increased islet mass in both the HFD/HFD and the CTR/HFD offspring. In-depth islet analysis of specific islet cells revealed an increase in β -cell area in both the HFD/HFD and the CTR/HFD offspring. CTR/HFD had a concomitant increase in β -cell area which served to normalize the β -cell ratio to control levels. In contrast, the HFD/HFD group exhibited a 40% increase in the β -cell ratio, which suggest a deregulation in the paracrine function of these islets. Deregulation of this interaction in the HFD/HFD group is likely to increase susceptibility to diabetes in later life. Nevertheless, short term dietary intervention at this early age does ameliorate the effects in the HFD/CTR group.

Sources of Research Support: U.S. National Institutes of Health Grants RO1DK079194 and R24DK090964 awarded to Kevin Grove, ONPRC.

Nothing to Disclose: SMC, JMB, DLT, ANC, MSS, KLG

Pub # OR01-4

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (11:15 AM-12:45 PM)

Title Cannabinoid 1 Receptor Blockade Improves Incretin-Mediated Insulin Secretion in Pancreatic Beta Cells

Author String W Kim, Z Liu, ME Doyle, Y-K Shin, OD Carlson, J Fiori, S Gadkaree, EK Lee, QZ Lao, JM Egan
NIA/NIH, Baltimore, MD; Johns Hopkins Medical Institutes, Baltimore, MD; NIA/NIH, Baltimore, MD

Body
OBJECTIVE-Cannabinoid 1 receptors (CB1Rs) are expressed in peripheral tissues, including islets of Langerhans, where their function(s) is under scrutiny. We have now investigated the role of CB1Rs in modulating incretin-mediated insulin secretion.
RESEARCH DESIGN AND METHODS-Using human and mouse islets, several β -cell lines and CB1R-null (*CB1R*^{-/-}) mice we investigated cannabinoids as regulators of adenylyl cyclase activity and insulin secretion in β cells. We also examined the effect of CB1R on insulin secretion and glucose clearance in vivo by performing an intraperitoneal glucose (1 g/kg body weight) tolerance test in *CB1R*^{+/+} and *CB1R*^{-/-} mice after infusion of the incretin GLP-1 (1.5 pmol/kg[\cdot min]).
RESULTS-In pancreatic β -cell lines, synthetic and endogenous CB1R agonists diminished incretin-mediated cAMP accumulation and insulin secretion, and genetic and pharmacological blockade of CB1R resulted in an increase of insulin secretion and improved glucose tolerance in response to a glucose load, when compared to control mice. Furthermore, *CB1R*^{-/-} mice showed greater glucose-dependent insulin secretion during infusion of GLP-1. Additionally, *CB1R*^{-/-} mice had increased glucokinase and glucose transporter 2 expression in β cells.
CONCLUSIONS-Our results suggest that CB1R signaling in pancreatic islets may be harnessed to improve β -cell glucose responsiveness and preserve β -cell function in type 2 diabetes. CB1R antagonists contribute to the physiological regulation of glucose homeostasis through inhibiting CB1Rs expressed in peripheral tissues. Now, our findings show that blocking peripheral CB1Rs, which has been shown to be beneficial in reducing cardiometabolic risk factors, would also be beneficial to β -cell function.

Nothing to Disclose: WK, ZL, MED, Y-KS, ODC, JF, SG, EKL, QZL, JME

Pub # OR01-5

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (11:15 AM-12:45 PM)

Title Pancreatic Progenitor Cell Lines Derived from Patients with Congenital Hyperinsulinism

Author String LE Eastwood, A Stevens, RM Shepherd, J Kim, L Zeef, M Skae, I Banerjee, C Hall, P Clayton, KE Cosgrove MJ Dunne
University of Manchester, Manchester, UK; Royal Manchester Children's Hospital, Manchester, UK

Body **Background:** Congenital Hyperinsulinism (CHI) is a potentially lethal, β -cell associated, disorder of the neonate characterised by severe hypoglycaemia. Here, we sought to establish cell lines from patients as the first step towards *in vitro* models of the disease.
Methods: With informed consent, we obtained post-operative resections of pancreatic tissue from four patients with CHI. Tissues were collagenase-treated and resulting cells maintained in culture under standard conditions. RT-PCR, exon microarray gene chips, immunocytochemistry and intracellular calcium (Ca^{2+}) microfluorimetry techniques were used to examine the molecular and physiological properties of the cell lines
Results: Genotyping revealed that all of the patients carried different mutations in the SUR1 gene, *ABCC8*. Cell lines continuously maintained over a 6 month period developed a mesenchyme to epithelial-like morphological transition. This involved a decrease in the protein expression of α -smooth muscle actin (α -sma) and vimentin and the upregulation of E-cadherin and occludin. After the transition, computational analysis of gene array data revealed a significant down regulation of α -sma/vimentin and upregulation of E-cadherin and claudin-1 ($p < 0.05$), and changes in the clustering of gene pathways ($p < 0.05$) consistent with a mesenchymal to epithelial transformation. Mapping the gene arrays by principal component analysis indicated all four cell lines had a high degree of similarity when maintained in continuous culture. In each of the cell lines, islet and pancreatic endocrine progenitor cell markers were identified and their expression was found to be stable over time. These included: *Pdx1*, *Sox9*, *Hlxb9*, *Nkx2.2*, *Nkx6.1*, *NeuroD1*, *Pax6* and *FoxA2*. Functional studies to monitor changes in cytosolic Ca^{2+} levels showed that, whilst ATP (0.1 mM) and histamine (0.1 mM) readily raised intracellular Ca^{2+} , each of the cells lines failed to consistently respond to acetylcholine (0.1 mM), depolarising concentrations of KCl (40 mM) and glucose (15 mM), $n=6$ in each case.
Conclusions: These data show that cell lines can be derived from CHI tissue and that they display markers of pancreatic progenitor cells associated with the secondary transition phase of pancreatic development. Cell lines expressed a relatively consistent gene array profile despite differences in the genetic basis of CHI, and each line exhibited a change in phenotype consistent with a mesenchyme to epithelial transition.

Sources of Research Support: Medical Research Council, UK.

Disclosures: PC: Consultant, Serono; Principal Investigator, Ipsen. Nothing to Disclose: LEE, AS, RMS, JK, LZ, MS, IB, CH, KEC, MJD

Pub # OR01-6
Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (11:15 AM-12:45 PM)
Title $G_{s\alpha}$ Deficiency in POMC Neurons Leads to Hyperglycemia with Reduced Serum Insulin Levels and Impaired Counterregulation to Hypoglycemia
Author String A Kablan, M Chen, O Gavrilova, LS Weinstein
NIDDK, NIH, Bethesda, MD; NIDDK, NIH, Bethesda, MD

Body The central nervous system (CNS) maintains glucose and energy homeostasis in response to hormonal and metabolic changes. One important regulatory mechanism involves sensing of ambient glucose levels by glucose-excited neurons (GE). One group of GE neurons is the proopiomelanocortin (POMC) neurons, which originate in the arcuate nucleus of the hypothalamus and project to many hypothalamic and extra-hypothalamic sites. α -MSH release from these neurons leads to reduced food intake, increased energy expenditure and direct changes in peripheral glucose metabolism. $G_{s\alpha}$ is a ubiquitously expressed G protein α -subunit that couples many receptors to intracellular cAMP generation. Observations in pseudohypoparathyroidism type 1A patients and various $G_{s\alpha}$ knockout mouse models have implicated CNS $G_{s\alpha}$ pathways in the regulation of energy and glucose metabolism. As the K_{ATP} channel is involved in glucose sensing in both pancreatic β cells and POMC neurons and $G_{s\alpha}$ /cAMP signaling is required for normal glucose sensing in β cells, we hypothesized that $G_{s\alpha}$ may also play a critical role in glucose sensing in POMC neurons. To test this we generated mice with $G_{s\alpha}$ deficiency in POMC neurons (POMCGsKO) by mating $G_{s\alpha}$ -floxed mice with POMC-cre mice. POMCGsKO mice were born at the expected Mendelian ratio and had normal body and composition on both regular and high fat diets. POMCGsKO mice (both males and females) developed hyperglycemia with significantly reduced serum insulin levels. Baseline phospho-Akt levels were reduced in POMCGsKO liver and muscle, consistent with the presence of lower insulin levels. These changes appeared to be due to an insulin secretion defect rather than a change in β cell mass or insulin production, as POMCGsKO mice had normal islet morphology and similar insulin and $G_{s\alpha}$ levels in β cells as compared to controls based on immunohistochemistry. Results of insulin tolerance tests showed that the initial hypoglycemic response to insulin was unaffected while the counterregulatory response to hypoglycemia was markedly impaired in POMCGsKO mice. These initial results suggest that loss of $G_{s\alpha}$ in POMC neurons leads to reduced insulin secretion in response to POMC neurons 'sensing' a lower glucose levels. In addition, these mice also appear to have a defect in sensing acute hypoglycemia resulting in an impaired counterregulatory response.

Sources of Research Support: NIDDK Intramural Research Program, NIH.

Nothing to Disclose: AK, MC, OG, LSW

Pub # OR02-1

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Bone, Calcitropic Hormones & Vitamin D (11:15 AM-12:45 PM)

Title Mammary-Specific Ablation of the Calcium-Sensing Receptor Gene Results in Transient Hypercalcemia and Hypercalciuria during Lactation

Author String R Mamillapalli, J VanHouten, P Dann, D Bikle, W Chang, D Shoback, J Wysolmerski
Yale University School of Medicine, New Haven, CT; VA Medical Center/University of California San Francisco, San Francisco, CA

Body The calcium-sensing receptor (CaSR) is expressed on mammary epithelial cells (MECs) during lactation. Pharmacological data suggested that activation of the CaSR on MECs inhibits the secretion of PTHrP into milk and the circulation, but stimulates the transport of calcium into milk. Global deletion of the CaSR results in severe hypercalcemia and early death. Therefore, in order to provide genetic support for the functions of the mammary gland CaSR, we ablated the CaSR gene specifically in MECs. Mice bearing a floxed CaSR gene (CaSR^{lox/lox}) were bred to mice expressing Cre recombinase driven by a milk protein gene (beta-lactoglobulin) promoter (BLG-Cre). In BLG-Cre/CaSR^{lox/lox} mice, the CaSR gene is disrupted only in MECs and only at the end of pregnancy. Deletion of the CaSR did not affect mammary alveolar development and BLG-Cre/CaSR^{lox/lox} mice lactated normally. Mammary CaSR mRNA levels were reduced from 60 to 95 percent in lactating BLG-Cre/CaSR^{lox/lox} mice. As expected, loss of the CaSR resulted in elevated levels of mammary gland PTHrP mRNA and milk PTHrP concentrations. Loss of the mammary CaSR also led to transient hypercalcemia. On day 2 of lactation, mean calcium levels in BLG-Cre/CaSR^{lox/lox} mice were 13.8±0.6 as compared to 10.7±0.3 in controls. However, on day 9 of lactation, circulating calcium concentrations were the same in each group. In contrast on day 9, urinary calcium excretion was 5 times higher in BLG-Cre/CaSR^{lox/lox} mice as compared to controls. Since other sites of CaSR expression remain intact in these mice, the transient nature of the hypercalcemia is likely due to stimulation of the renal CaSR and compensatory hypercalciuria. These data demonstrate that the mammary CaSR regulates systemic calcium metabolism during lactation.

Sources of Research Support: NIH R01 CA153702.

Nothing to Disclose: RM, JV, PD, DB, WC, DS, JW

Pub # OR02-2

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Bone, Calcitropic Hormones & Vitamin D (11:15 AM-12:45 PM)

Title Biphasic and Dosage-Dependent Regulation of Osteoclastogenesis by β -Catenin

Author String Y Wan, W Wei, D Zeve, X Wang, Y Du, JM Suh, JE Zerwekh, PC Dechow, JM Graff
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Body Wnt/ β -catenin signaling is a critical regulator of skeletal physiology. However, previous studies mainly focus on its roles in osteoblasts; while its specific function in osteoclasts is unknown. This is a clinically important question because neutralizing antibodies against Wnt antagonists are promising new drugs for bone diseases. Here we show that, in osteoclastogenesis, β -catenin is induced during MCSF-mediated quiescence-to-proliferation switch, but suppressed during RANKL-mediated proliferation-to-differentiation switch. Genetically, β -catenin deletion blocks osteoclast precursor proliferation, while β -catenin constitutive activation sustains proliferation but prevents osteoclast differentiation, both causing osteopetrosis. In contrast, β -catenin heterozygosity enhances osteoclast differentiation, causing osteoporosis. Biochemically, Wnt activation attenuates whereas Wnt inhibition enhances osteoclastogenesis. Mechanistically, β -catenin activation increases GATA2/Evi1 expression but blocks RANKL-induced c-Jun phosphorylation. Therefore, β -catenin exerts a pivotal biphasic and dosage-dependent regulation of osteoclastogenesis. Importantly, these findings suggest that Wnt activation is a more effective treatment for skeletal fragility than previously recognized that confers anabolic and anti-catabolic dual benefit.

Sources of Research Support: University of Texas Southwestern Medical Center Endowed Scholar Startup Fund (Y.W.), a BD Biosciences Research Grant Award (Y.W.), CPRIT grant (RP100841, Y.W.), NIH grants (R01 DK066556, R01 DK064261 and R01 DK088220, J.M.G.), predoctoral fellowship (D.Z.) from American Heart Association South Central Affiliate. Y.W. is a Virginia Murchison Linthicum Scholar in Medical Research.

Nothing to Disclose: YW, WW, DZ, XW, YD, JMS, JEZ, PCD, JMG

Pub # OR02-3

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Bone, Calcitropic Hormones & Vitamin D (11:15 AM-12:45 PM)

Title Sclerostin Stimulates Osteocyte Support of Osteoclast Activity by a RANKL-Dependent Pathway

Author String GJ Atkins, M Kogawa, DM Findlay, AR Wijenayaka
The University of Adelaide, Adelaide, Australia

Body The *SOST* gene product, sclerostin, is produced by mature osteocytes embedded in mineralised bone and is a negative regulator of bone mass and osteoblast differentiation (1, 2). While it is clear that sclerostin has an anti-anabolic role, we hypothesised that sclerostin also has a direct catabolic activity. In support of this, both human (3) and animal (4) studies employing a neutralising anti-sclerostin antibody observed decreases in bone resorption parameters. To test this hypothesis we treated human primary pre-osteocyte cultures, cells we have found are exquisitely sensitive to sclerostin (2), or mouse osteocyte-like MLO-Y4 cells, with recombinant human sclerostin (rhSCL) and measured effects on pro-catabolic gene expression. Sclerostin dose-dependently up-regulated expression of RANKL mRNA and down-regulated that of OPG mRNA, causing an increase in the RANKL:OPG mRNA ratio. To examine the effects of rhSCL on resulting osteoclastic activity, MLO-Y4 cells plated onto a bone-like substrate were primed with rhSCL for 3 days and then either mouse splenocytes or human peripheral blood-derived mononuclear cells (PBMC) were added. This resulted in cultures with elevated osteoclastic resorption (approximately 7-fold). The increased resorption was abolished by co-addition of recombinant OPG. Interestingly, the numbers of tartrate resistant acid phosphatase (TRAP)-positive multinucleated cells formed was not affected by rhSCL treatment, but remained basally high. rhSCL had no effect on TRAP-positive cell formation from monocultures of either splenocytes or PBMC. Together, these results suggest that sclerostin may have a catabolic action through promotion of osteoclast formation and activity by osteocytes in a RANKL-dependent manner. These novel findings may help explain the dramatic increase in bone mass observed when *SOST* is mutated in humans or sclerostin is neutralized in animal models, in addition to its anti-anabolic actions.

(1) Paszty C, et al 2010 J Bone Miner Res 25:1897-1904

(2) Atkins GJ, et al 2011 Sclerostin is a locally acting regulator of late-osteoblast/pre-osteocyte differentiation and regulates mineralization through a MEPE-ASARM dependent mechanism. J Bone Miner Res (In Press)

(3) Padhi D, et al 2011 J Bone Miner Res 26:19-26

(4) Li X, et al 2009 J Bone Miner Res 24:578-588

Sources of Research Support: National Health and Medical Research Council of Australia Project Grant Scheme.

Nothing to Disclose: GJA, MK, DMF, ARW

Pub # OR02-4

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Bone, Calcitropic Hormones & Vitamin D (11:15 AM-12:45 PM)

Title Humanin Protects Mice from Dexamethasone-Induced Bone Growth Impairment

Author String F Zaman, D Chrysis, B Fadeel, L Savendahl
Karolinska Institutet, Stockholm, Sweden; University of Patras, Patras, Greece; Karolinska Institutet, Stockholm, Sweden

Body BACKGROUND: Glucocorticoids (GCs) are commonly used to treat multiple disorders including cancers and many inflammatory conditions. Besides the desired treatment effect, GCs cause a multitude of side-effects including bone growth impairment in children. We have previously shown that GCs induce apoptosis in growth plate chondrocytes. So far, no treatment has been identified preventing GC-induced bone growth impairment. We hypothesized that a derivative of humanin, [Gly(14)] (HNG) may prevent GC-induced apoptosis in chondrocytes and if so, could have the capacity to rescue bone growth during GC treatment. METHODS: We used an array of model systems including ATDC-5 chondrocytes, cultured fetal rat metatarsal bones, cultured human growth plate cartilage, human monocyte-derived macrophages (HMDMs), and mice (FVB and C57BL). Animals were subcutaneously injected with dexamethasone (Dexa; 2mg/kg/day), alone or in combination with intraperitoneal HNG (1[μ]g/mice/day) for four weeks and femur growth was monitored by X-ray. RESULTS: In vivo studies in mice confirmed that Dexa significantly impairs bone growth. In contrast, bone growth was completely rescued in animals treated with the combination of Dexa and HNG. Furthermore, Dexa increased chondrocyte apoptosis in growth plate cartilage of treated mice, whereas HNG+Dexa significantly prevented chondrocytes from undergoing apoptosis. The anti-apoptotic effect of HNG was confirmed in cultured human growth plate biopsies exposed to Dexa. Fetal rat metatarsal bones cultured with Dexa alone or in combination with HNG showed that HNG rescues metatarsal bone growth during Dexa exposure, suggesting a local action in the growth plate. To rule out if HNG may interfere with the desired anti-inflammatory effect of Dexa, HMDMs were exposed to lipopolysaccharide (LPS; 1ng/ml) and treated with Dexa (1[μ]M) alone or in combination with HNG (10nM). As expected, Dexa significantly blocked the release of TNF-alpha. When LPS-exposed HMDMs were treated with HNG+Dexa, the release of TNF-alpha was further decreased, suggesting that HNG does not interfere with the anti-inflammatory effect of Dexa. CONCLUSION: The humanin derivative HNG efficiently rescues growth plate cartilage and bone growth during Dexa treatment. Our data suggest a novel treatment strategy based on the administration of HNG in combination with GCs which could reduce negative side-effects in bone and potentially in other tissues as well in patients receiving GCs.

Sources of Research Support: S[au]llskapet Barnav[ar]rd, Stockholm, Sweden; Stiftelsen Frimurare Barnhuset i Stockholm, Sweden; Barncancer fonden, Sweden.

Nothing to Disclose: FZ, DC, BF, LS

Pub # OR02-5

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Bone, Calcitropic Hormones & Vitamin D (11:15 AM-12:45 PM)

Title Deubiquitination Regulates PTHR Fate after Ligand Stimulation

Author String V Alonso, C Magyar, B Wang, PA Friedman
University of Pittsburgh School of Medicine, Pittsburgh, PA; UCLA, Los Angeles, CA

Body Renal failure and secondary hyperparathyroidism are accompanied by PTH resistance and downregulation of the type I PTH receptor (PTH1R). As with most GPCRs, activating ligands, PTH(1-84), PTH(1-34) and non-activating ligands, PTH(7-84), PTH(7-34), induce PTHR internalization in kidney and osteoblast cells. However, the mechanism that regulates the sorting of internalized receptors between recycling and degradation pathways is not fully understood. We now report a basic mechanism that explains the PTH1R fate after PTH stimulation in rat osteosarcoma cells. PTH1R recycles faster after challenge with PTH(1-34) than with PTH(7-34). More than 50% of the receptor recycles by 30 min after treatment with PTH(1-34). In contrast, after challenge with PTH(7-34), less than 50% of the receptor recycles by 1 hr. The receptor is fully recycled by 2 hr after PTH(1-34) stimulation. However, the recycling of PTH1R is incomplete at this time in cells treated with PTH(7-34). We observed by immunoblot that PTH(7-34) induces PTH1R downregulation, and that this decrease in PTH1R protein expression was abolished by the proteasome inhibitor MG-132. These data suggest that the slower and incomplete recycling induced by PTH(7-34) is a proteasome-dependent mechanism. We studied receptor ubiquitination as a pathway leading to proteasome degradation. Both PTH(1-34) and PTH(7-34) induced PTHR Lys48-polyubiquitination, corresponding to proteasome 26S subunit targeting. Notably, ubiquitination by PTH(1-34) was transient and started to diminish within 1h of stimulation with only slight residual PTH1R ubiquitination at 2 hr. However, receptor ubiquitination following PTH(7-34) remained elevated and sustained after 2 hr. We further observed that PTH(1-34) but not PTH(7-34) stimulated an increase in the PTH1R-specific deubiquitinating enzyme USP2. Overexpression of USP2 prevented PTH(7-34)-induced PTH1R degradation. We conclude that PTH(1-34) promotes coupled PTH1R ubiquitination and deubiquitination, whereas PTH(7-34) activates only ubiquitination, thereby leading to PTH1R downregulation. These findings may explain PTH resistance in diseases associated with elevated PTH(7-84) levels.

Sources of Research Support: NIH R01DK 054171.

Nothing to Disclose: VA, CM, BW, PAF

Pub # OR02-6

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Bone, Calcitropic Hormones & Vitamin D (11:15 AM-12:45 PM)

Title A Key Role for CARM1 Arginine Specific Methylation in Vitamin D Receptor Mediated Transcription

Author String LJ Mady, Y Zhong, P Dhawan, S Christakos
UMDNJ - New Jersey Medical School, Newark, NJ

Body Hormone dependent transcriptional regulation involves chromatin remodeling by coactivator proteins. Although roles of acetylation and phosphorylation in chromatin remodeling have been widely studied, recent findings have also indicated an important role for methylation. Little is known however about the exact role of methyltransferases and their regulation by different physiological signaling pathways. Using renal proximal or distal convoluted tubule cells or VDR transfected COS-7 cells and the rat 24(OH)ase promoter we found that CARM1 (a methyltransferase that methylates histone 3 at arginine 17) cooperates with the p160 coactivator GRIP1 to enhance 1,25(OH)₂D₃ induced transcription. Transfection of GRIP1 enhanced 1,25(OH)₂D₃ induced transcription 2 fold and co-expression of GRIP + CARM1 resulted in a 4-9 fold enhancement. All activity was dependent on 1,25(OH)₂D₃. A CARM1 mutant lacking methyltransferase activity failed to enhance 24(OH)ase activity in cooperation with GRIP1. In addition, when the GRIP1 mutant ([Delta]AD2) which lacks the binding site for CARM1 was used, cooperative activation was also not observed. This result supports a role for GRIP1 as a primary coactivator or bridge to recruit the secondary coactivator, CARM1. Thus, the coactivator function of CARM1 requires methyltransferase activity and coexpression of GRIP1. When PRMT2A, another arginine methyltransferase, was substituted for CARM1, cooperative transactivation with GRIP1 was not observed, suggesting a preferential role of CARM1 in VDR transactivation. Moreover, through ChIP-seq analysis we identified genome wide co-occurrence of VDR binding and histone 3 arginine 17 methylation in kidney cells in response to 1,25(OH)₂D₃, further supporting a fundamental role of CARM1 in VDR mediated transcription. In addition to 24(OH)ase, CARM1 co-activator activity was also observed for 1,25(OH)₂D₃ induction of TRPV6 and osteopontin. However, we found that cAMP or calcitonin transcriptional induction of 1 α (OH)ase (involved in the synthesis of 1,25(OH)₂D₃) is inhibited by CARM1 in proximal convoluted tubule cells. Thus CARM1 can act as an activator or repressor, depending on the context of transcription factors at a specific promoter. Our findings indicate for the first time that CARM1 methylation may have a broad yet fundamental role in modulating target genes associated with the vitamin D endocrine system and thus in the control of the biological function of vitamin D.

Nothing to Disclose: LJM, YZ, PD, SC

Pub #	M1
Session Information	MEET-THE-PROFESSOR: CLINICAL - Delayed Puberty (11:15 AM - 12:00 PM)
Title	Delayed Puberty
Author String	PB Kaplowitz Children's National Medical Center, Washington, DC
Body	Nothing to Disclose: PBK

Pub # OR03-1

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Factor Cascades Regulating Pituitary Development & Tumorigenesis (11:15 AM-12:45 PM)

Title Persistent Expression of Activated Notch Inhibits Corticotrope and Melanotrope Differentiation and Results in Dysfunction of the HPA Axis

Author String LB Goldberg, LT Raetzman
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Body Concerted development of the hypothalamic-pituitary-adrenal (HPA) axis is necessary for normal stress response and loss of functional proopiomelanocortin (POMC) can lead to adrenal insufficiency, hypoglycemia and increased risk for obesity in humans. The Notch signaling pathway is known to play a key role in pituitary corticotrope development and cell differentiation, a key part of the HPA axis. Notch signaling components are present during pituitary development and their expression diminishes as cell differentiation proceeds. Studies have shown that global loss of the Notch effector *Hes1* accelerates the differentiation of corticotropes, suggesting Notch signaling is necessary to prevent early differentiation of these cells. In order to address whether Notch is sufficient to prevent differentiation of melanotropes and corticotropes, cre recombinase, under the control of the *Pomc* promoter, was used to express activated *Notch1*. At embryonic day 16.5 (e16.5) and postnatal day 1 (p1), control (Wt) and conditional Notch1 transgenic (Tg) pituitaries appear histologically similar. However, at these same ages, POMC expression is observed in corticotropes and melanotropes of Wt mice, but is nearly absent in both cell types in Tg mice. At p1, nearly all cells of the intermediate lobe (IL) of Tg mice express Sox2, a marker of pituitary progenitors, whereas only a thin layer of cells lining the lumen of the pituitary are Sox2-positive in Wt mice. In order to determine the mechanism by which differentiation is inhibited, quantitative RT-PCR of multiple transcription factors necessary for the differentiation of POMC-expressing cells was performed. At e16.5, *Tpit*, *NeuroD1*, and *Mash1* mRNA levels are significantly decreased in Tg animals as compared to Wt littermates. Furthermore, by p30, melanotropes and corticotropes aberrantly expressing *Notch1* have been largely eliminated. Consistent with loss of POMC in the pituitary, the adrenals of Tg mice are hypoplastic and stress-induced corticosterone levels are reduced as compared to Wt. This study shows that Notch signaling is sufficient to prevent the differentiation of POMC-expressing cells and that properly timed Notch signaling is necessary for HPA axis differentiation and function.

Sources of Research Support: RO1 DK076647A to LTR.

Nothing to Disclose: LBG, LTR

Pub # OR03-2

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Factor Cascades Regulating Pituitary Development & Tumorigenesis (11:15 AM-12:45 PM)

Title Hypothyroidism Induced by ISL1 Inactivation in Thyrotrophs

Author String F Castinetti, ML Brinkmeier, KR Vella, AN Hollenberg, L Gan, SA Camper
University of Michigan, Ann Arbor, MI; Beth Israel Deaconess Medical Center, Harvard, Boston, MA;
University of Rochester, Rochester, NY

Body ISL1 is a LIM homeodomain transcription factor necessary for development of the heart, pancreas, and retina. *Isl1* deficient mice (*Isl1*^{-/-}) die early during embryogenesis (e10.5) due to heart defects, and at that time, they have an undersized pituitary primordium. ISL1 exhibits dynamic expression in the pituitary from early embryogenesis to adulthood. Here we report the cell specific expression of ISL1 and assessment of its role in differentiated gonadotrophs and thyrotrophs. We used a *Tshb* BAC transgene recombineered with cre to generate mice with *Isl1* deleted in thyrotrophs: *Isl1*^{flox/flox};Tg(*Tshb-cre*). These mice have growth insufficiency, and TSH levels are in the normal range. Circulating T4 levels are decreased, and their thyroid glands are significantly smaller. The presence of low T4 levels without increased pituitary TSH production is consistent with decreased thyrotroph function. The expression of several transcription factors with roles in early pituitary development, including *Pitx2*, *Pitx1*, *Lhx3* and *Lhx4*, are significantly decreased in *Isl1*^{flox/flox};Tg(*Tshb-cre*) pituitary glands, suggesting that *Isl1* is a master regulator. To challenge the thyrotroph function of *Isl1*^{flox/flox};Tg(*Tshb-cre*) mice, we treated them for 4 weeks with a low iodine diet enriched with the anti-thyroid drug, propyl-thio-uracil. This induces low T4 levels and elevated TSH response in normal mice, but the *Isl1*^{flox/flox};Tg(*Tshb-cre*) mice have a severely blunted TSH response. In contrast, deletion of *Isl1* in gonadotrophs with an *Lhb-cre* transgene has no obvious effect on gonadotroph function or fertility. These results show that ISL1 is necessary for thyrotroph function, in addition to its role in development of Rathke's pouch.

Sources of Research Support: NICHD 34283 awarded to SAC.

Nothing to Disclose: FC, MLB, KRV, ANH, LG, SAC

Pub # OR03-3

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Factor Cascades Regulating Pituitary Development & Tumorigenesis (11:15 AM-12:45 PM)

Title The Wnt/ β -Catenin Effector Tcf3 Is Required for Normal Hypothalamic-Pituitary Development

Author String C Gaston-Massuet, M Koniordou, C Andoniadou, M McCabe, S Jayakody, P Le Tissier, M Dattani, JP Martinez-Barbera
Institute of Child Health, University College London, London, UK; Insitute of Child Health, University College London, London, UK; National Institute for Medical Research, London, UK

Body Aberrant development of the pituitary gland can result in the clinical manifestation of hypopituitarism. The Wnt/b-catenin pathway has been shown to be involved in normal organogenesis and terminal differentiation of the pituitary gland, and when this pathway is deregulated can cause pituitary tumours. However, the specific developmental role of some of the Wnt/b-catenin effectors, such as Tcf3 in the pituitary has been hampered due to the early lethality of Tcf3 null embryos. We have generated *Tcf3^{Fl/-};Hesx1^{Cre/+}* mice and embryos, which lack Tcf3 in the Hesx1-expressing cells within the early forebrain and pituitary gland. A low proportion of *Tcf3^{Fl/-};Hesx1^{Cre/+}* animals exhibit dwarfism indicating that deficiency in Tcf3 can lead to hypopituitarism in mice. In agreement with this notion, analysis of *Tcf3^{Fl/-};Hesx1^{Cre/+}* mutant embryos reveals hyperplasia of the pituitary gland, possibly due to both the expansion of early specified Rathke's pouch (RP) epithelium and increased proliferation of RP progenitors at mid gestation. *Tcf3^{Fl/-};Hesx1^{Cre/+}* embryos show upregulation of Wnt/b-catenin canonical downstream targets and a low proportion of mutants exhibit disrupted terminal differentiation of hormone-producing cells, which could explain the hypopituitarism observed in postnatal life. We also present data supporting a genetic interaction between Tcf3 and Hesx1, which suggests that both factors may exert similar molecular functions. Together these data demonstrate for the first time an essential role for Tcf3 in pituitary development and place Tcf3 as a candidate gene that may be mutated in human endocrinopathies.

Sources of Research Support: BRC ICH, National Institute for Health Research; Wellcome Trust.

Nothing to Disclose: CG-M, MK, CA, MM, SJ, PLT, MD, JPM-B

Pub # OR03-4

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Factor Cascades Regulating Pituitary Development & Tumorigenesis (11:15 AM-12:45 PM)

Title P53-Dependent Suppression of Pituitary Tumor Growth in Nude Mice by MEG3 Non-Coding RNA

Author String Y Zhou, Y Nakayama, CS De La Hoz Ulloa, KA Rice, X Zhang, A Klibanski
Massachusetts General Hospital and Harvard Medical School, Boston, MA

Body *Maternally expressed gene 3 (MEG3)* is an imprinted gene belonging to the *DLK1-MEG3* locus located on human chromosome 14q32. Its imprinting is regulated by an intergenic differentially methylated region (IG-DMR). Our previous studies demonstrated that MEG3 is highly expressed in normal human pituitary, but undetectable in clinically non-functioning pituitary adenomas (NFAs) of gonadotroph origin. The loss of MEG3 expression is at least in part attributed to hypermethylation of its promoter and the IG-DMR. We have demonstrated that MEG3, functioning as a non-coding RNA, inhibits *in vitro* cell proliferation, activates tumor suppressor p53 and selectively regulates p53-target genes. *Meg3* gene deletion in mice caused embryonic developmental defects and perinatal death. These data strongly suggest that MEG3 functions as a novel non-coding RNA tumor suppressor in the pituitary. We therefore used PDFS cells, which are derived from a human NFA and do not express MEG3, to test tumor suppression by MEG3. MEG3 expression was re established in PDFS cells by stable transfection of expression vectors in which MEG3 cDNA was controlled by a CMV promoter (CMV-MEG3) or by a Tet on promoter (Tet-MEG3). These clones were inoculated into nude mice to test their tumorigenicity. Tumor formation from PDFS-CMV-MEG3 cells in nude mice was significantly delayed compared to tumors formed from the parental PDFS cells or PDFS-CMV-MEG3-del5 expressing a known mutant MEG3. PDFS-Tet-MEG3 cells grew significantly fewer and smaller tumors in mice fed with water containing Dox, which induces MEG3 expression, than in mice fed with water without Dox. To investigate the mechanism by which MEG3 suppresses tumor growth, p53 expression was knocked down in PDFS-Tet-MEG3 cells by RNA interference to generate PDFS-Tet-MEG3-p53kd. In nude mice, PDFS-Tet-MEG3-p53kd cells rapidly grew tumors regardless of MEG3 induction by Dox. In contrast, MEG3 induction by Dox significantly suppressed tumor formation from PDFS-Tet-MEG3-NSi cells which expressed a non-specific small hairpin RNA. Taken together, these data indicate that MEG3 suppresses pituitary tumor growth induced from a human NFA derived cell line in nude mice and this tumor suppression is mediated by p53. Our data support the hypothesis that loss of MEG3 expression plays a major role in development of human NFAs.

Sources of Research Support: Grants from the National Institutes of Health (A. Klibanski, R01DK40947), the Guthart Family Foundation and the Jarislowsky Foundation.

Nothing to Disclose: YZ, YN, CSDLHU, KAR, XZ, AK

Pub # OR03-5

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Factor Cascades Regulating Pituitary Development & Tumorigenesis (11:15 AM-12:45 PM)

Title Filamin-A Is Essential for the Regulation of Dopamine D2 Receptor Expression and Signaling in Tumoral Lactotroph Cells

Author String G Mantovani, E Peverelli, E Vitali, S Ferrero, E Laws, A Villa, P Beck-Peccoz, A Spada, AG Lania Endocrine Unit, Fondazione IRCCS Ospedale Maggiore, Milano, Italy; Endocrine Unit, IRCCS Istituto Clinico Humanitas, Rozzano (Milano), Italy; Pathology, Fondazione IRCCS Ospedale Maggiore, Milano, Italy; Brigham and Women's Hospital, Boston, MA; Universit[agrave] di Milano Bicocca, Monza, Italy

Body Dopamine agonists (DA) are the first choice treatment of prolactinomas. However, a subset of patients is resistant to DA, due to still undefined alterations in D2 receptor (D2R) expression or signaling. Recently, in other cell systems D2R was found to associate with filamin-A (FLNA), a widely expressed cytoskeleton protein with scaffolding properties, that affects receptor trafficking and signaling. The aim of this study was to investigate the role of FLNA on D2R expression and signaling in prolactinomas and MMQ cells. 17 prolactinomas were examined for FLNA expression by immunohistochemistry. Cultured cells obtained from 4 prolactinomas and MMQ cells were transfected with negative control siRNA or Flna siRNA. In these cells we evaluated D2R expression and targeting to cell membrane and D2R-mediated antisecretory and antiproliferative pathways. Tumor DNA was screened for mutations in D2R and FLNA interacting regions and methylation defects in their promoters. Immunohistochemistry demonstrated reduced FLNA expression in resistant prolactinomas with respect to DA-responsive adenomas. Silencing the expression of FLNA in sensitive prolactinomas and MMQ cells reduced D2R expression by 58±9% and 65±12% reduction respectively, and totally abrogated the DA-induced inhibition of PRL release and generation of antiproliferative signals, i.e. extracellular signal-regulated kinase (ERK1/2) pathway. Moreover, in tumor tissues no mutation in the interacting regions of D2R and FLNA genes nor alterations in methylation pattern of their promoters were detected. Our data indicate that FLNA is crucial for D2R expression and signaling in lactotrophs. We suggest that the impaired response to DA may be related to the reduction of FLNA expression in resistant prolactinomas.

Nothing to Disclose: GM, EP, EV, SF, EL, AV, PB-P, AS, AGL

Pub # OR03-6

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Factor Cascades Regulating Pituitary Development & Tumorigenesis (11:15 AM-12:45 PM)

Title Up-Regulation of Histone-Modifying Enzymes: Implications in the Pathogenesis of Human Pituitary Adenomas

Author String IR Thompson, D Tang, S Xu, ME Wierman, ER Laws, Y Shi, RS Carroll, UB Kaiser
Division of Endocrinology, Diabetes and Hypertension, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; Endocrinology, Metabolism and Diabetes, University of Colorado-Denver, Aurora, CO; Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Body The pathogenesis of pituitary adenomas is poorly understood. Epigenetic alterations, including changes in histone modification and DNA methylation at promoter CpG islands, can result in silencing of key tumor suppressor genes. Recent evidence has emerged that menin acts as a tumor suppressor in endocrine tissues, including the pituitary, by associating with histone methyltransferase complexes to activate p18 and p21 cyclin-dependent kinase (CDK) inhibitors. Lysine-specific demethylase 1 (LSD1) and SMCX/Jarid1C regulate histone H3K4 methylation, an epigenetic marker for gene activation. LSD1 has been functionally implicated in pituitary cell specification and physiology. In addition, LSD1 and Jarid1 family demethylases are aberrantly regulated in several human cancers; hence, we investigated their involvement in pituitary adenoma pathogenesis. We hypothesized that changes in the expression levels of oncogenes or tumor suppressor genes during pituitary adenoma development could be the result of either altered gene expression or protein levels of these epigenetic factors. Through the Brigham and Women's Hospital tissue bank, we obtained pituitary adenoma specimens, including 6 non-functional, 2 somatotrope-, and 1 thyrotrope-derived tumors. Normal control pituitary tissue samples were acquired at the time of autopsy. Both RNA and protein were isolated from tumors and normal tissue and were subjected to RT-qPCR and SDS-PAGE for RNA and protein analysis, respectively. SMCX, LSD1 and LSD2 mRNA levels were all significantly higher within the adenoma subset, compared to the control samples. In contrast, by Western blot analysis, elevated protein levels were observed only for SMCX. The effect of the LSD inhibitor, tranylcypromine, on the proliferation of the rat GH3 somatolactotrope-derived cell line was also investigated. A dose-dependent decrease in GH3 cell proliferation, as determined by MTS assay, was observed in the presence of tranylcypromine after 48 to 96 h. In conclusion, SMCX is elevated at both RNA and protein levels in pituitary adenoma samples compared to normal pituitary controls, suggesting a role for H3K4 demethylation in pituitary tumor pathogenesis, perhaps through regulation of oncogenes. This role of H3K4 demethylation is further supported by evidence that inhibition of histone demethylases reduces cell proliferation in a pituitary cell line.

Nothing to Disclose: IRT, DT, SX, MEW, ERL, YS, RSC, UBK

Pub # M2
Session Information MEET-THE-PROFESSOR: CLINICAL - Difficult Diabetes Cases (11:15 AM - 12:00 PM)
Title Difficult Diabetes Cases
Author String GT McMahon
Brigham & Women's Hospital, Boston, MA
Body Session supported by: Lilly USA, LLC and Merck & Co., Inc.
Nothing to Disclose: GTM

Pub # OR04-1

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Lipids in Health & Disease (11:15 AM-12:45 PM)

Title Nicotine Induces Oxidative Stress and Exacerbates High Fat Diet (HFD)-Induced Hepatic Steatosis in a Mouse Model of Diet-Induced Obesity

Author String M Mangubat, A Lyzlov, I Nzenwa, R Shen, AP Sinha Hikim, TC Friedman, I Sinha-Hikim
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Body **Background and Objective:** Smoking is a major risk factor for diabetes, cardiovascular disease, chronic obstructive pulmonary disease and lung cancer. There is evidence that smoking may contribute to non alcoholic fatty liver disease (NAFLD). NAFLD has also been identified as an independent risk factor for atherosclerosis and cardiovascular disease. The health risk associated with smoking is exaggerated by obesity and is the leading causes of morbidity and mortality worldwide. We hypothesize that nicotine plus a high-fat diet (HFD) will have additive effects on the severity of hepatic steatosis in obese mice.

Experimental Design: Adult C57BL6 male mice were fed with normal diet (ND) or HFD with 60% of calories derived from fat with twice daily injections of nicotine (1.5 mg/kg BW, ip) or saline for 10 weeks.

Results: Compared with mice on ND, mice fed with HFD exhibited significant weight gain and increased abdominal fat. Nicotine-treated mice on a HFD showed significantly less weight gain than mice fed with HFD plus saline. High resolution light and electron microscopy revealed increased lipid accumulation of varying sizes with a decrease in the amount of endoplasmic reticulum and mitochondria in hepatocytes from mice on HFD; these changes were not seen in normal hepatocytes. Addition of nicotine to HFD resulted in a further increase in lipid accumulation and in the size of lipid droplets, in the incidence of hepatocyte apoptosis (characterized by TUNEL as well as by electron microscopy), with a diminution of cellular organelles. Mice treated with both nicotine and HFD had greater oxidative stress, indicated by low hepatic GSH/GSSG ratio (9.8 ± 1.7), relative to mice on HFD alone (14.3 ± 1.6). The increased incidence of apoptosis and oxidative stress in the combined treatment group was accompanied by activation of c-Jun-NH₂-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) and perturbation of the BAX/BCL-2 ratio. Co-staining for TUNEL and phospho-JNK or phospho-p38 MAPK further confirmed kinase activation only in those hepatocytes undergoing apoptosis. **Conclusion:** Combined treatments of nicotine and HFD trigger greater oxidative stress, activate JNK and p38 MAPK signaling, sensitize hepatocytes to apoptosis and further worsen HFD-induced hepatic steatosis. We surmise that nicotine plus HFD is likely to be a very toxic combination in patients. Thus, smokers, especially those who eat a high fat diet, should not be liver donors.

Sources of Research Support: NIH-NCCR Grant # U54 RR026138-01 (KCN) at Charles Drew University.

Nothing to Disclose: MM, AL, IN, RS, APSH, TCF, IS-H

Pub # OR04-2
Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Lipids in Health & Disease (11:15 AM-12:45 PM)
Title Central Cellular Leptin Resistance Can Manifest as Hepatic Steatosis Independent of Obesity
Author String JP Warne, F Alemi, AS Reed, H Chan, ML Piper, ME Mullin, MG Myers, Jr, CU Corvera, AW Xu
University of California San Francisco, San Francisco, CA; San Francisco VA Medical Center, San Francisco CA; University of Michigan Medical School, Ann Arbor, MI

Body Common obesity is associated with the diminished ability of leptin to stimulate signal transduction pathways, such as those utilizing signal transducer and activator of transcription-3 (STAT3) and phosphatidylinositol 3-kinase (PI3K), in neurons expressing the long form of the leptin receptor (LepRb). Such diminished leptin signaling, here termed cellular leptin resistance, can impact the ability of leptin to modulate metabolism. Indeed, we and others (1,2) have previously shown that impaired leptin-stimulated STAT3 signaling results in hyperphagia and profound obesity. In this study, we show that low dose (4[μ g/day) leptin administration into the lateral ventricle of the adult mouse brain over 7 days significantly reduces liver triglyceride levels without affecting feeding and body adiposity. In order to explore if signaling through PI3K is required for this effect of leptin, leptin-induced PI3K signaling was attenuated by moderate transgenic over-expression of phosphatase and tensin homolog (PTEN) in LepRb-expressing neurons. This transgenic approach results in mice with ~50% reduction in basal PI3K signaling and diminished ability of leptin to signal through PI3K, without affecting basal or leptin-stimulated STAT3 signaling. Mice with reduced PI3K signaling exclusively in LepRb neurons exhibit an increase in the mRNA expression of genes involved in liver *de novo* lipogenesis and an increase in hepatic triglyceride levels without significant effect on body weight, adiposity, hepatic insulin signaling or hepatic triglyceride release when compared to control littermates. Simultaneous downregulation of PI3K and STAT3 function in LepRb-expressing neurons does not cause further change in adiposity, when compared to mice with STAT3 deletion alone, but results in more severe hepatic steatosis. Increased feeding and body adiposity are commonly considered to be the primary outcomes of leptin resistance with hepatic steatosis presumed to be a secondary consequence of obesity. However, our results strongly suggest that central cellular leptin resistance, when manifested as impaired leptin-induced PI3K signaling, can result in hepatic steatosis independent of hyperphagia and obesity.

(1) Bates SH et al., Nature 2003; 421:856

(2) Piper ML et al., Mol Endocrinol 2008; 22:751

Sources of Research Support: NIH grant R01DK080427 awarded to AWX; NIH Pediatric Endocrine Training grant T32DK07161 awarded to ASR; NIH DERC P30 DK063720 supported core facilities.

Nothing to Disclose: JPW, FA, ASR, HC, MLP, MEM, MGM, CUC, AWX

Pub # OR04-3

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Lipids in Health & Disease (11:15 AM-12:45 PM)

Title Testosterone Inhibits mRNA Expression of Fatty Acid Synthase in the Liver and Protects Against Hepatic Steatosis in Tfm Mouse

Author String V Muraleedharan, S Akhtar, D Kelly, KS Channer, HT Jones
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Body A clinical feature of insulin resistance is the development of fatty liver (hepatic steatosis). Testosterone deficiency is common in men with type 2 diabetes and is associated with insulin resistance. The testicular feminized mouse (Tfm) model exhibits a non-functional androgen receptor (AR) and low circulating testosterone. We have previously shown that a high cholesterol/fat diet promotes atherosclerosis and hepatic steatosis in the Tfm mouse. Testosterone replacement therapy (TRT) prevents Hepatic steatosis and reduces fatty streak formation^{1, 2}. This effect is in part independent of the AR, the exact mechanism being unclear. We have therefore investigated the effect of TRT on key enzymes involved with fatty acid metabolism. Tfm mice were fed a high cholesterol diet *ad libitum* for 28 weeks and received either physiological testosterone replacement (intramuscular mixed testosterone esters, Sustanon 100[Ograve], 25mg/kg -Tfm+T n=12) or placebo (saline -Tfm+P n=12) and were compared to placebo treated wild type littermate controls (XY+P n=12). The liver tissue was collected, snap frozen in liquid nitrogen and the relative concentrations of mRNA were analysed by qPCR (QRT-PCR, SYBR Green) for target genes Fatty acid synthase(FAS), Acetyl-CoA carboxylase(ACC), Lipoprotein Lipase(LPL), Hormone sensitive lipase(HSL), PPAR α and PPAR γ

Results

There was a significant increase in the relative expression of FAS (Relative fold change in mRNA ($2^{-[\Delta\Delta Ct]}$) Mean \pm SD 11.4 ± 17 $p=0.049$) and ACC (2.5 ± 2.2 $p=0.041$) in the Tfm mouse compared with their XY littermates. Following testosterone treatment, mRNA expression was significantly decreased for FAS (3 ± 3 $p=0.018$) and showed a non-significant decrease in ACC (1.3 ± 1 $p=0.42$). HSL showed a significant reduction in the Tfm mouse after treatment with TRT (0.77 ± 0.41 $p=0.018$). But there was no significant change between placebo treated wild type and Tfm (1.3 ± 0.6 $p=0.15$). PPAR γ , PPAR α , receptors and LPL did not show any significant changes in the three groups.

Discussion

Expression of mRNA of ACC and FAS, the first two enzymes in FA synthesis, were greater in the liver from Tfm compared to the wild type. TRT significantly suppressed FAS mRNA but not ACC mRNA. This suggests that testosterone has an action on FAS which is in part independent of the AR and may contribute to the protective effect on hepatic steatosis occurring in the T treated Tfm.

1. Nettleship, J. E., T. H. Jones, et al. (2007). *Circulation* 116(21): 2427-2434

2. Dugdale, C., Nettleship, J. and Jones, TH (2009). *Endocrine Abstracts* (2009) 19 P329

Nothing to Disclose: VM, SA, DK, KSC, HTJ

Pub # OR04-4

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Lipids in Health & Disease (11:15 AM-12:45 PM)

Title Increased Capacity of Distal Intestinal Epithelium to Transport Long-Chain Fatty Acids in Obese Mice Fed a High-Fat Diet

Author String MD Lynes, R Fogley, EP Widmaier
Boston University, Boston, MA

Body The mechanisms by which diets that are high in saturated fat (HSF) contribute to obesity are uncertain. We previously demonstrated that chronic HSF leads to insulin resistance, hyperleptinemia, and obesity even when the total fat content (%) of the diet is normal, and when energy consumption is unchanged compared to controls on a normal-fat diet. HSF also increased expression of the fatty acid transport protein CD36 as well as global intestinal alkaline phosphatase (gIAP) in, specifically, the distal regions of the small intestine. The two proteins coimmunoprecipitated, and gIAP dephosphorylated phospho-CD36, suggesting gIAP plays a role in regulating the phosphorylation status and therefore the transport activity of CD36. Here, the hypothesis that these HSF-induced gene expression changes lead to functional changes in distal portions of the small intestine that may be consistent with the advent of obesity was tested in male C57Bl/6 mice. Isolated murine intestinal (jejunal) epithelial cells treated with intestinal alkaline phosphatase (IAP) demonstrated a dose-dependent increase in the initial rate of uptake of a fluorescently-labeled long chain fatty acid (LCFA) analog. This increase was blocked by treatment with the irreversible CD36-specific inhibitor sulfosuccinimidyl oleate (SSO). Pretreatment of isolated enterocytes with IAP or SSO had no effect on uptake of a short chain fatty acid analog. Chronic HSF resulted in obesity after several weeks, as expected, and also increased the CD36-dependent uptake of the LCFA analog in isolated jejunal enterocytes compared to cells from animals fed a diet low in saturated fat. We conclude that regulation of CD36 activity by intestinal alkaline phosphatase is a mechanism for modulating intestinal epithelial LCFA uptake. In mice, increased expression of CD36 in parallel with its putative regulating enzyme gIAP may serve to adapt the distal small intestine to HSF diets such that LCFA uptake is accelerated; this, in turn, may contribute to increased adiposity and the subsequent endocrine and metabolic sequelae of obesity.

Sources of Research Support: NSF grant IOS-0446057 awarded to EPW, and a grant from the Boston University Undergraduate Research Opportunities Program awarded to RF.

Nothing to Disclose: MDL, RF, EPW

Pub # OR04-5

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Lipids in Health & Disease (11:15 AM-12:45 PM)

Title Hepatic Leptin Signaling Is Required for Leptin to Normalize Lipid Metabolism in Leptin-Deficient Mice

Author String FK Huynh, SD Covey, TJ Kieffer
University of British Columbia, Vancouver, Canada

Body

BACKGROUND:
The hormone leptin regulates body weight by centrally reducing food intake and increasing energy expenditure. However, leptin can also affect metabolism independent of its central effects on body weight by acting directly on the liver. Recently, we showed that a chronic loss of hepatic leptin signaling *in vivo* resulted in increased hepatic insulin sensitivity and improved glucose tolerance. The literature, however, suggests that acute versus chronic hepatic leptin signaling may have differential effects on hepatic insulin sensitivity. Thus, we sought to determine the effects of acute hepatic leptin signaling on metabolism *in vivo*.

METHODS:
The Cre-*lox* approach was used to generate transgenic mice lacking hepatic leptin receptors, which were then bred onto a leptin-deficient *ob/ob* background. To determine the effects of acute hepatic leptin signaling, these mice were given leptin therapy and then aspects of glucose and lipid metabolism were assessed.

RESULTS:
Upon leptin treatment, *ob/ob* mice without functional hepatic leptin receptors had similar body weight, food intake, plasma cholesterol, and free fatty acid levels to non-transgenic *ob/ob* controls. Similar to mice with a chronic, life-long loss of hepatic leptin signaling, leptin-treated *ob/ob* mice lacking hepatic leptin signaling also had increased insulin sensitivity. Further, in response to leptin therapy, *ob/ob* mice lacking liver leptin signaling had elevated fasting triglyceride levels and impaired lipid tolerance compared to *ob/ob* mice with fully intact leptin signaling. The effects of leptin on metabolism persisted even after leptin therapy was ceased, indicating possible long-term effects of leptin therapy.

CONCLUSIONS:
Leptin acts as a negative regulator of insulin signaling in the liver and plays a role in regulating triglyceride clearance. Thus, acute hepatic leptin action profoundly affects glucose and lipid metabolism, both of which are dysregulated in type 2 diabetes. Collectively, our results suggest that impaired hepatic leptin action may contribute to the aberrant metabolism seen in type 2 diabetes.

Sources of Research Support: Natural Sciences and Engineering Research Council of Canada; Canadian Institutes for Health Research; Michael Smith Foundation for Health Research.

Nothing to Disclose: FKH, SDC, TJK

Pub # OR04-6

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Lipids in Health & Disease (11:15 AM-12:45 PM)

Title Substitution of Standard Soybean Oil with Olive Oil-Based Lipid Emulsion in Parenteral Nutrition: Comparison of Vascular, Metabolic and Inflammatory Effects

Author String D Smiley, J Siqueira, A Gosmanov, C Newton, L Peng, P Chandra, T Ziegler, G Umpierrez
Emory University School of Medicine, Atlanta, GA; Emory Rollins School of Public Health, Atlanta, GA

Body Soybean oil-based lipid emulsions are the only FDA-approved lipid formulation for clinical use in parenteral nutrition (PN). Recently, concerns with its use have been raised due to pro-inflammatory effects that may lead to increased complications, as they are rich in [omega]-6 polyunsaturated fatty acids. This prospective, randomized, cross-over study compared the vascular, metabolic, immune and inflammatory effects of 24h-infusions of soybean oil-based PN (Intralipid[Ograve]), olive oil-based PN (ClinOleic [Ograve]), lipid-free PN, and normal saline in 12 healthy subjects. We examined vascular [blood pressure (BP), heart rate, and endothelial function by flow-mediated dilatation (FMD)], metabolic [glucose, insulin, c-peptide, lipids and free fatty acids (FFAs) and euglycemic hyperinsulinemic clamp], inflammatory markers (CRP, TNF-alpha, IL-6), oxidative stress markers (cystine, cysteine, glutathione, glutathione disulfide), immune function (phagocytic and oxidative burst activity of monocytes and granulocytes), and autonomic nervous system activity by **heart rate variability (HRV)**. Subjects [41±7 yrs, BMI 32±2 kg/m²] were normotensive, nonsmokers, and had normal glucose tolerance by OGTT. **Soybean oil-PN increased systolic BP compared to olive oil-PN (p<0.05). Soybean oil-PN significantly reduced FMD from baseline (-23% at 4 h and -25% at 24 h, both p<0.01); in contrast, olive oil-PN, lipid free-PN, and saline did not change FMD.** Differences in FMD between groups were significantly different between **soybean oil-based and olive oil-based PN** after 24 hours (**p= 0.02**). **Compared to saline, soybean oil-PN, olive oil-PN, and lipid free-PN similarly increased** glucose, insulin and C-peptide concentrations during infusion (p<0.05). There were no significant changes in insulin sensitivity by euglycemic clamp, FFAs, lipid profile, inflammatory and oxidative stress markers, immune function parameters, or sympathetic activity between **soybean oil and olive oil-based lipid emulsions**. **In summary, the 24h-infusion of PN containing soybean oil-based lipid emulsion increased BP and impaired endothelial function compared to PN containing olive oil-based lipid emulsion and lipid-free PN in healthy subjects.** These vascular changes may have significant implications in worsening outcome in subjects receiving nutrition support. Randomized controlled trials with relevant clinical outcome measures are needed in patients receiving PN with olive oil-based and soybean oil-based lipid emulsions.

Sources of Research Support: Baxter Pharmaceuticals.

Disclosures: GU: Principal Investigator, Baxter Pharmaceuticals. Nothing to Disclose: DS, JS, AG, CN, LP, PC, TZ

Pub # M3

Session Information MEET-THE-PROFESSOR: CLINICAL - Drug Therapies for Osteoporosis: When, Which & How Long To Use? (11:15 AM - 12:00 PM)

Title Drug Therapies for Osteoporosis: When, Which & How Long To Use?

Author String AJ De Santis
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Body Session supported by: Amgen

Nothing to Disclose: AJDS

Pub # OR05-1

Session Information ORAL SESSION: TRANSLATIONAL - Genetics & Epigenetics in GnRH Regulation (11:15 AM-12:45 PM)

Title Normosmic Congenital Hypogonadotropic Hypogonadism Due to TAC3/TACR3 Mutations: Prevalence, Characterization of Neuroendocrine Phenotypes and Novel Mutations

Author String B Francou, B Bouligand, A Voican, L Amazit, S Trabado, J Fagart, G Meduri, S Brailly-Tabard, P Chanson, J Lecomte, A Guiochon-Mantel, J Young
Assistance Publique-H[ocirc]pitaux de Paris, H[ocirc]pital Bic[ecirc]tre, Le Kremlin-Bic[ecirc]tre, France; Assistance Publique-H[ocirc]pitaux de Paris, H[ocirc]pital Bic[ecirc]tre, Le Kremlin-Bic[ecirc]tre, France; University Paris-Sud, Faculté de Médecine Paris-Sud, Le Kremlin-Bic[ecirc]tre, France; H[ocirc]pital CHU de Tours, Tours, France

Body Context: TAC3/TACR3 mutations have been reported in normosmic, non syndromic congenital hypogonadotropic hypogonadism (nCHH). In the absence of animal models, studies of human neuroendocrine phenotypes associated with neurokinin B and NK3R receptor dysfunction can help to decipher the pathophysiology of this signaling pathway.
Objective: To characterize novel TACR3 mutations and to analyze neuroendocrine profiles in nCHH patients with TAC3/TACR3 biallelic mutations.
Results: From a cohort of 352 CHH, we excluded patients with Kallmann syndrome, selected 173 nCHH patients and identified eight TACR3 mutations (1 frameshift, 4 missense and 3 nonsense mutation) in 5 adults (4 sporadic cases, 1 familial)(5.2% of our nCHH population). Molecular analyses, modeling and functional studies demonstrated the pathogenic nature of four novel mutations. Three patients with TAC3/TACR3 biallelic mutations had an apulsatile LH profile and low-frequency alpha-subunit pulses. Using the same assays, we found a statistically significant higher mean FSH/LH ratio in 11 patients with TAC3/TACR3 biallelic mutations than in nCHH patients with biallelic mutations in GPR54/KISS1R (n=4), GNRH1 (n=2) or GNRHR (n=11), and nCHH patients with no identified mutations (n=32) or in patients with Kallmann syndrome and mutations in KAL1 (n=19), FGFR1 (n=17) or PROKR2/PROKR2 (n=14) ($p < 0.0001$). Pulsatile GnRH administration to three patients harboring TAC3/TACR3 mutations increased alpha-subunit pulsatile frequency and reduced the FSH/LH ratio.
Conclusion: The gonadotropin axis dysfunction associated with nCHH due to TAC3/TACR3 mutations is related to a low GnRH pulsatile frequency leading to a low frequency of alpha-subunit pulses and to an elevated FSH/LH ratio. We propose that this ratio is useful for pre-screening nCHH patients for TAC3/TACR3 mutations.

Sources of Research Support: ANR Kalgenopath, INSERM, Univ Paris Sud, FRM.

Nothing to Disclose: BF, BB, AV, LA, ST, JF, GM, SB-T, PC, PL, AG-M, JY

Pub # OR05-2

Session Information ORAL SESSION: TRANSLATIONAL - Genetics & Epigenetics in GnRH Regulation (11:15 AM-12:45 PM)

Title Three Mutations in TACR3 Identified in Patients with GnRH Deficiency Disrupt NK3R Function through Distinct Mechanisms

Author String SD Noel, AP Abreu, S Xu, AC Latronico, SB Seminara, R Carroll, UB Kaiser
Brigham and Women's Hospital and Harvard Medical School, Boston, MA; FMUSP, São Paulo, Brazil; Massachusetts General Hospital, Boston, MA

Body Previously, our group identified three loss-of-function mutations in TACR3, encoding NK3R, in a cohort of patients with GnRH deficiency; one heterozygous mutation, R295S, located in the third intracellular loop (IL3) and two homozygous mutations, Y256H and Y315C, in the 5th and 6th transmembrane (TM5 and TM6) domains respectively. We investigated the mechanisms through which these mutations affect NK3R function by performing receptor expression, ligand binding and intracellular signaling assays in COS-7 cells transfected with WT or mutant NK3Rs. Western blot analysis demonstrated that the NK3R mutants R295S and Y315C had cell surface receptor levels similar to WT, but Y256H had markedly reduced levels, indicating that loss of function of NK3R in patients with this mutation is due to impaired receptor expression. Next, ligand binding assays were performed using ¹²⁵I-NKB and unlabeled NKB to produce competition binding curves. R295S bound ¹²⁵I-NKB similarly to WT, but Y315C failed to show appreciable binding. As Y315C is expressed at the cell surface, its impaired binding suggests that the mutation leads to a conformational change in the receptor that prevents ligand binding. The Y256H also failed to bind ¹²⁵I-NKB, consistent with its lack of cell surface expression. Finally, the effects on intracellular signaling were analyzed by measuring inositol phosphate (IP) accumulation. As expected, both Y256H and Y315C failed to stimulate IP accumulation compared to WT. Interestingly, R295S also failed to stimulate IP accumulation, indicating that while this mutant is expressed at the cell surface and can bind NKB, it cannot activate Gq protein-mediated signaling. In addition, we found that WT NK3R activated the MAPK pathway, as measured by NKB-stimulated ERK phosphorylation, whereas this activation was blunted for R295S. As R295S was a heterozygous mutation, we tested a potential dominant negative effect on WT receptor signaling by co-transfecting varying ratios of WT and R295S expression vectors into COS-7 cells and measuring IP accumulation. Co-transfection of WT and R295S expression vectors resulted in reduced IP accumulation compared to WT alone, supporting a dominant negative effect. Collectively, our data show that mutations in different domains of NK3R impair receptor function through distinct mechanisms and further indicate roles for IL3 in receptor signaling, for TM5 in receptor folding and trafficking to the plasma membrane, and for TM6 in ligand binding.

Nothing to Disclose: SDN, APA, SX, ACL, SBS, RC, UBK

Pub # OR05-3

Session Information ORAL SESSION: TRANSLATIONAL - Genetics & Epigenetics in GnRH Regulation (11:15 AM-12:45 PM)

Title Kallmann Syndrome Due to a Novel *FGFR1* Gene Mutation

Author String A Flint, S Witchel, W Chemaitilly
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Body **Background:** Kallmann syndrome (KS) is the association of hypogonadotropic hypogonadism and anosmia/hyposmia. Failure of proper migration of olfactory nerves leads to agenesis of the olfactory bulbs and lack of penetration of GnRH neurons into the hypothalamus. Patients with KS often present with delayed sexual maturation and may recognize deficits in smell. KS is associated with mutations in the *KAL1* gene located at Xp22.3 and in the *FGFR1* gene at 8p11.2-p11.1. Other genes associated with KS include *PROKR2*, *PROKR2*, *FGF8*, *NELF*, and *CHD7* (1). Phenotypic heterogeneity is common even within a single family. We report a novel *FGFR1* mutation associated with a spectrum of phenotypes.

Clinical Case: A 15-year-old male presented to Pediatric Endocrinology for evaluation of pubertal delay. Healthy, with a reportedly normal sense of smell, he had growth deceleration and was found to have small testes measuring 2.5 cm with Tanner stage III pubic hair. Studies revealed prepubertal levels of FSH 2.6 mIU/mL (normal 2.0-9.2 mIU/mL), LH 0.4 mIU/mL (normal 0.4-7.0 mIU/mL) and testosterone of 10.3 ng/dL (normal 200-620 ng/dL). His bone age was delayed at 13 years and 6 months. Family history was notable for KS with normosmia in the proband's mother. The proband's twin brother was undergoing normal puberty. A maternal aunt, her son, and the maternal grandfather reported anosmia with normal fertility; two maternal uncles were infertile. Testing using the University of Pennsylvania Smell Identification Test confirmed anosmia in the proband's maternal aunt and her son and hyposmia (6th percentile for age) in the proband. The proband's mother and twin brother had normosmia.

Genotyping for KS in the proband revealed a previously unreported heterozygous IVS7-1 G>T mutation in the *FGFR1* gene. This mutation is predicted to generate a splicing defect due to interruption of the canonical splice acceptor site in intron 7.

Conclusions: The novel mutation reported here is likely to be the cause of the proband's phenotype since a dysfunctional gene product is predicted based on the mutation identified in the *FGFR1* gene. This family is also interesting due to the phenotypic spectrum. Phenotypic heterogeneity appears to be common in KS and may reflect the diverse oligogenic nature of this disorder (2). Thus, a thorough family history should be obtained in all patients with pubertal delay and a diagnosis of KS should not be ruled out merely due to reported normosmia in the patient in question.

(1) Balasubramanian R et al., *Neuroendocrinology* 2010; 92:81-99
(2) Sykiotis GP et al., *Proc Natl Acad Sci U S A* 2010; 107:15140-4

Nothing to Disclose: AF, SW, WC

Pub # OR05-4

Session Information ORAL SESSION: TRANSLATIONAL - Genetics & Epigenetics in GnRH Regulation (11:15 AM-12:45 PM)

Title Gain-of-Function Mutation in FGFR1 in Human GnRH Deficiency

Author String H Miraoui, KW Keefe, G Sykiotis, L Plummer, T Raivio, AA Dwyer, Y Sidis, P Tsai, M Mohammadi, N Pitteloud
Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland; Massachusetts General Hospital, Boston, MA; University of Patras Medical School, Patras, Greece; Institute of Biomedicine, University of Helsinki, Helsinki, Finland; University of Colorado, Boulder, CO; New York University School of Medicine, New York, NY

Body **Background:** Congenital human gonadotropin-releasing hormone (GnRH) deficiency results from defects in GnRH neuronal development or GnRH secretion/action. Patients present with hypogonadotropic hypogonadism (HH), absent puberty, infertility, and a sense of smell that is either normal (normosmic idiopathic HH, nHH) or absent (Kallmann syndrome, KS). Germline mutations in fibroblast growth factor receptor 1 (*FGFR1*) underlie both nHH and KS. *FGFR1* has 2 major alternative splice isoforms, (*FGFR1-IIIa* & *FGFR1-IIIc*), expressed in epithelial and mesenchymal tissues, respectively, which differ in the 3rd immunoglobulin-like domain and have divergent ligand specificities. To date, mutations in only the *FGFR1-IIIc* isoform have been linked to human GnRH deficiency.

Methods: Two GnRH-deficient patients carrying *FGFR1-IIIb* rare sequence variants were studied. Functionality of the novel *FGFR1-IIIb* mutations was studied using: i) Structural modeling; ii) FGF10-induced receptor signaling activity in a luciferase reporter assay; iii) Assays of receptor protein expression and maturation.

Results: Two novel heterozygous *FGFR1-IIIb* mutations (p.H313Y, p.T357I) were identified in female KS patients with absent puberty, and were absent from 200 ethnically-matched controls. The patient harboring p.H313Y also exhibits bilateral hearing loss and skeletal phenotypes with agenesis of multiple teeth and camptodactyly. Both mutations impair receptor function in vitro: T357I showed gain-of-function with ligand-independent signaling and increased total yet decreased cell-surface expression. H313Y showed mild loss-of-function with decreased response to FGF10 stimulation, decreased cell-surface expression, and maturation defects.

Conclusion: The identification of *FGFR1-IIIb* isoform mutations associated with human GnRH deficiency and olfactory defects suggests an important role for mesenchyme-epithelial interaction in GnRH ontogeny. Further, *FGFR1-IIIb* T357I is the first gain-of-function mutation demonstrated in human GnRH deficiency, given its low expression at the cell surface, this mutant might signal from an intracellular compartment.

Nothing to Disclose: HM, KWK, GS, LP, TR, AAD, YS, PT, MM, NP

Pub # OR05-5
Session Information ORAL SESSION: TRANSLATIONAL - Genetics & Epigenetics in GnRH Regulation (11:15 AM-12:45 PM)
Title Epigenetic Regulation of Sexually Dimorphic Kiss1 Neurons in the Hypothalamus of Male and Female Mice
Author String SJ Semaan, AS Kauffman
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Body The neuropeptide kisspeptin, encoded by the *Kiss1* gene, is an important regulator of GnRH neurons. In the rodent hypothalamus, *Kiss1* neurons are present in the arcuate (ARC) nucleus and the anatomical continuum comprising the anteroventral periventricular (AVPV) and anterior periventricular (PeN) nuclei. Interestingly, females have more AVPV/PeN *Kiss1* neurons than males and this sexual dimorphism may underlie the ability of females, but not males, to produce a preovulatory LH surge. We have shown that sex steroids during the postnatal critical period direct the sexual differentiation of AVPV/PeN *Kiss1* cells, but the mechanism(s) by which postnatal hormones guide this process is unknown. We recently determined that sexual differentiation of the AVPV/PeN *Kiss1* population is not dependent on BAX-mediated apoptosis. Thus, adult males do not have fewer *Kiss1* neurons because of higher *Kiss1* cell death during development. Here we investigated whether epigenetic mechanisms during early development influence sexually dimorphic *Kiss1* gene expression. The most common mechanisms for epigenetic alterations in gene expression are histone acetylation (generally associated with increased gene expression) and DNA methylation (usually associated with decreased gene expression). Thus, we tested whether either or both of these epigenetic mechanisms contributes to the *Kiss1* sex difference in the AVPV/PeN. We first administered a histone deacetylase (HDAC) inhibitor, valproic acid (VPA), or vehicle to male and female mice during the postnatal critical period, when sexual differentiation of the *Kiss1* system is known to occur. The brains of these mice were examined in adulthood for AVPV/PeN *Kiss1* expression. Although AVPV *Kiss1* cell numbers were higher in VPA-treated males and females than vehicle-treated mice, postnatal VPA treatment did not eliminate the AVPV/PeN *Kiss1* sex difference in adulthood. Thus, histone acetylation may modulate development of *Kiss1* cells in the AVPV in both sexes, but is unlikely to generate the *Kiss1* sex difference. Next, we investigated whether specific regions of the *Kiss1* gene are differentially methylated in the AVPV of adult males and females. Our preliminary data indicate that some sites on the *Kiss1* gene are differentially methylated between males and females, correlating with the sex difference in AVPV *Kiss1* expression. These findings suggest that epigenetic processes may contribute to the development of *Kiss1* neurons in mice.

Sources of Research Support: NIH 00 HD056157; R01 HD065856; U54 HD012303; F32 HD066849; NSF IOS-1025893.

Nothing to Disclose: SJS, ASK

Pub # OR05-6

Session Information ORAL SESSION: TRANSLATIONAL - Genetics & Epigenetics in GnRH Regulation (11:15 AM-12:45 PM)

Title Rare Nucleotide Variants in *KISS1* in Patients with GnRH-Deficient Phenotypes

Author String Y-M Chan, R Lapatto, M Au, V Hughes, SDC Bianco, L Min, L Plummer, F Cerrato, A de Guillebon, F Wahab, A Dwyer, SE Kirsch, R Quinton, TD Cheetham, M Ozata, SB Ten, J-P Chanoine, N Pitteloud, WF Crowley, KA Martin, R Schiffmann, JE Hall, UB Kaiser, SB Seminara
 Massachusetts General Hospital, Boston, MA; Children's Hospital Boston, Boston, MA; Brigham and Women's Hospital, Boston, MA; Hospital for Sick Children, Toronto, Canada; Institute of Human Genetics, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne, UK; Gulhane School of Medicine, Ankara, Turkey; Maimonides Infants and Children's Hospital, Brooklyn, NY; University of British Columbia, Vancouver, Canada; Baylor Research Institute, Dallas, TX

Body *Background:* Loss-of-function mutations in the kisspeptin receptor in both humans and mice result in failure of sexual maturation and infertility due to GnRH deficiency. Similarly, deletion of kisspeptin in mice causes abnormal sexual maturation and hypogonadotropic hypogonadism. Thus, the gene encoding kisspeptin, *KISS1*, is a compelling candidate gene for isolated GnRH deficiency in humans. However, no inactivating mutations in *KISS1* have been reported to date.

Methods: The three exons of *KISS1* were sequenced in 888 probands with GnRH-deficient phenotypes (388 normosmic idiopathic hypogonadotropic hypogonadism [nIHH], 371 Kallmann syndrome, 79 hypothalamic amenorrhea, 18 men with testosterone >100 ng/dL but <270 ng/dL, 50 constitutional delay of puberty), as well as 188 controls. Additionally, 100 base pairs 5[prime] of the *KISS1* transcriptional start site were sequenced in 281 probands with nIHH. The effects of rare nucleotide variants were assessed *in silico* and *in vitro*.

Results: Thirteen probands harbored 9 heterozygous rare nucleotide variants in *KISS1* not seen in control patients: g.1-3659C>T (in 2 patients), c.1-7C>T (in 2 patients), p.G35S, p.C53R, p.S77I, p.Q82K, p.P110T (in 3 patients), p.F117L, and p.A129V. Of the 4 variants that lie within the mature kisspeptin peptide (p.S77I, p.Q82K, p.P110T & p.F117L), p.F117L was found to impair kisspeptin receptor activation as measured by inositol phosphate (IP₃) generation *in vitro*. The S77I variant, which did not impact IP₃ generation, was noted to lie within a putative PEST domain and may have effects on the stability of kisspeptin. Three variants lie within the coding region but outside of the mature kisspeptin peptide (p.G35S, p.C53R & p.A129V). The variant p.G35S (c.103G>A) alters the final nucleotide of exon 2 and is predicted to disrupt splicing. The p.C53R variant is predicted *in silico* to be deleterious. Two rare variants lie outside of the coding region: g.1-3659C>T lies within a cyclic-AMP response element and impairs transcription *in vitro*, and c.1-7C>T lies within the consensus Kozak sequence, a sequence that enhances translational efficiency.

Conclusions: Rare nucleotide variants in *KISS1* are found in patients with GnRH-deficient states. Some impair *KISS1* function through a variety of mechanisms. These heterozygous variants may work synergistically with other genetic and/or environmental factors to cause abnormalities in puberty and reproductive function.

Nothing to Disclose: Y-MC, RL, MA, VH, SDCB, LM, LP, FC, AdG, FW, AD, SEK, RQ, TDC, MO, SBT, J-PC, NP, WFC, KAM, RS, JEH, UBK, SBS

Pub # OR06-1

Session Information ORAL SESSION: TRANSLATIONAL - Steroidal Regulation of Breast & Prostate Cancer (11:15 AM-12:45 PM)

Title Aryl Hydrocarbon Receptor Modulation of Estrogen Receptor-alpha-Mediated Gene Regulation by a Multimeric Chromatin Complex Involving the Two Receptors and the Coregulator RIP140

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Body Although crosstalk between *Aryl Hydrocarbon Receptor* (AhR) and *Estrogen Receptor-alpha* (ER α) is well established, the mechanistic basis and the involvement of other proteins in this process are not known. Because we observed an enrichment of AhR binding motifs in the regulatory regions of many ER α regulated genes, we investigated how AhR might modulate ER α -mediated gene transcription in breast cancer cells. Gene regulations were categorized into groups based on their pattern of stimulation in the presence of estradiol (E2) and/or dioxin and were denoted E2 stimulated, dioxin stimulated or dual ligand stimulated. ER α , AhR, *Aryl Hydrocarbon Receptor Translocator* (ARNT), and *Receptor Interacting Protein 140* (RIP140) were recruited to gene regulatory regions in a gene-specific and E2/dioxin ligand-specific manner. Knock-down of AhR markedly increased the expression of ER-mediated genes upon E2 treatment. This was not attributable to a change in the level of ER α or recruitment of ER α or phosphoSer5-RNA Pol II to the gene regulatory sites, but rather was associated with a greatly diminished recruitment of the coregulator RIP140 to gene regulatory regions. Studies changing the cellular level of RIP140 revealed coactivator or corepressor roles for this coregulator in E2 and dioxin-mediated gene regulation, the choice of which was determined by the presence or absence of ER α at the gene regulatory sites. Coimmunoprecipitation and ChIP-reChIP studies documented that E2 treatment of cells promoted formation of a multimeric complex of ER α , AhR and RIP140 at the ER α binding sites of regulated genes. Our findings highlight a novel mechanism by which AhR controls, through switching the function of the coregulator RIP140 from a coactivator to a corepressor, the kinetics and magnitude of ER α -mediated gene stimulation. The observations support the idea that gene-specific regulation of transcription requires an exquisite combination of stimuli, receptors, DNA binding elements, and coregulators to obtain a cellular response that is fine tuned and appropriately controlled in its magnitude and duration.

Sources of Research Support: Grants from The Breast Cancer Research Foundation (BSK), and the NIH (P01AG024387 BSK and T32ES007326 ZME).

Nothing to Disclose: ZM-E, BSK

Pub # OR06-2
Session Information ORAL SESSION: TRANSLATIONAL - Steroidal Regulation of Breast & Prostate Cancer (11:15 AM-12:45 PM)
Title A Humanized Pattern of Distribution of Aromatase Expression Causes Mammary Hyperplasia in Mice
Author String H Zhao, EK Pearson, D Chen, JS Coon V, DC Brooks, T Kurita, M Demura, FJ DeMayo, SE Bulun
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Body Aromatase enzyme that converts androgens to estrogens in peripheral tissues and breast is the key target of aromatase inhibitors, the most popular hormonal treatment of postmenopausal breast cancer. No *in vivo* experimental model of this treatment strategy has been available, however, partly because the tissue distribution patterns of aromatase expression in humans and mice are drastically distinct. In female mice, aromatase is expressed only in ovaries and the brain via 2 promoters, whereas women use at least 10 distinct promoters to express aromatase in many peripheral tissues including breast fat. To study the effects of aromatase and its inhibitors on breast, we recently generated 2 transgenic humanized aromatase (Arom^{hum}) mouse lines containing a single copy of the entire human aromatase gene. These novel Arom^{hum} female mice mimicked human physiology with respect to estrogen production and expressed the aromatase gene in many peripheral tissues-including breast fat-under the control of alternatively used human promoters. In female Arom^{hum} mice, the human aromatase gene was expressed at basal levels via the TNF-regulated distal promoter I.4 in the breast tissue. Immunohistochemistry localized aromatase to the adipose fibroblasts and myoepithelial cells in breast tissue of Arom^{hum} mice. There were no difference in the circulating levels of estradiol between the female Arom^{hum} mice and wild-type mice. The Arom^{hum} mice, however, exhibited much higher local estrogen concentrations in breast tissue leading to development of breast hyperplasia at 24 weeks. Cell proliferation evaluated by Ki67 staining was increased significantly by 4.8-fold in mammary epithelium of Arom^{hum} transgenic mice as compared to wild-type controls. In conclusion, humanized aromatase expression in the mammary gland driven by a set of human promoters caused mammary hyperplasia via accelerated epithelial proliferation. This new mouse model is the first of its kind and will be extremely valuable for studying the *in vivo* effects of altered metabolic states (e.g., obesity) or side effect of aromatase inhibitors.

Nothing to Disclose: HZ, EKP, DC, JSCV, DCB, TK, MD, FJD, SEB

Pub # OR06-3

Session Information ORAL SESSION: TRANSLATIONAL - Steroidal Regulation of Breast & Prostate Cancer (11:15 AM-12:45 PM)

Title Evidence for Androgenic Inhibition of Growth in Cultured Human Breast Tumor Tissues Despite a Decrease in the Ratio of Androgen Receptor to Estrogen Receptor Alpha with the Development of Breast Cancer

Author String TE Hickey, S Jindall, SN Birrell, LM Butler, WD Tilley
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Body
Background: Androgen receptor (AR) signaling exerts a growth inhibitory influence in normal breast tissue and this role may be sustained in estrogen receptor alpha (ER α) positive breast cancer. To date, the level and activity of AR in normal human breast tissues has not been well characterized or compared to malignant breast tissues. In addition, knowledge of AR action in breast cancer is largely based on cell lines. Therefore, in this study we compared relative levels of AR and ER α proteins in normal and malignant human breast tissues and determined the influence of androgen treatment on proliferation of epithelial or tumor cells in a complex tissue context.
Methods: Breast tissues were collected following surgery for benign breast disease or breast cancer, and were either fixed immediately for immunohistochemical analyses or cut into 3mm³ pieces and cultured in triplicate on gelatin sponges soaked in culture media containing 10% steroid-stripped FCS. Cultured tissues were treated for 96 hours as follows: control (vehicle); 5 α -dihydrotestosterone (DHT; 10nM); estradiol (E2; 10nM) or DHT+E2, with addition of BrdU at 48 hours to allow assessment of de novo DNA synthesis. Tissue sections were exposed to antisera to AR, ER α or BrdU and the percent of positive epithelial or tumor cells assessed by video image analysis (AR, ER α) or manual scoring (BrdU).
Results: Normal breast tissue from women with breast cancer (n = 38) had higher ER α levels (p < 0.01) but similar AR levels compared to normal tissue from women with benign breast disease (n = 23). Breast tumor tissues (n = 32) had elevated AR (p < 0.001) and ER α (p < 0.01) levels compared to normal tissues from both sources. The relative ratio of AR to ER α positive cells was higher in normal tissues from women without cancer (median 6:1) compared to normal tissues from women with cancer (median 3:1; p < 0.01) and breast tumor tissues (median 1:1; p < 0.001). Treatment with DHT significantly inhibited proliferation in cultured pieces of normal breast tissue (n = 6) and in ER α positive tumor tissue (n = 6), independent of the presence of E2 (p < 0.01 compared to control and DHT+E2).
Conclusion: AR levels increase concomitantly with ER α levels in the development of breast cancer, but AR loses predominance over ER α in the process. Despite loss of AR predominance, androgen retains the capacity to inhibit proliferation in ER β positive breast tumors.

Nothing to Disclose: TEH, SJ, SNB, LMB, WDT

Pub # OR06-4

Session Information ORAL SESSION: TRANSLATIONAL - Steroidal Regulation of Breast & Prostate Cancer (11:15 AM-12:45 PM)

Title The Insulin Resistance Grb14 Adaptor Protein Plays a Pivotal Role in Breast Cancer Development and Progression

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Body Grb14 is the most recent adaptor protein to join the Grb7/10/14 family that possess a unique *Between Plekstrin and SH2 (BPS)* domain. Grb14 competes with the phosphatase PTP1b for binding to the activated kinase domain of the insulin receptor. Such competition protects the kinase loop from dephosphorylation, thus enhancing IR autophosphorylation. Grb14 inhibits insulin-stimulated metabolic signals including glucose uptake and glycogen synthesis. However, this adaptor and the IR are also over-expressed in breast cancer where they have been associated with prognostic markers of disease behavior through unclear mechanisms. Using MDA-231 breast cancer cells, we show here that Grb14 promotes IR phosphorylation at the kinase Y1158/1162 and also at the IRS1 interaction Y972 sites. Grb14 also induces IRS1 expression and promotes Akt activation in these cells. To study functional relevance we used the polyoma middle T (PyMT) mouse model where mammary expression of the oncoprotein mimics several features of the human form of the disease. Grb14 inactivation significantly delayed the characteristic breast tumor development at 3 and 4 months. The number of carcinoma-affected breasts was also significantly reduced in PyMT/Grb14^{-/-} mice compared to PyMT/Grb14^{+/+} animals and the average total wet weight of all mammary tumors was also significantly decreased in PyMT/Grb14^{-/-} mice. Moreover, 5 of 20 PyMT/Grb14^{+/+} mice but only 2 of 20 PyMT/Grb14^{-/-} mice developed lung metastases. Our studies uncover Grb14 as an important IR signaling modifier in breast cancer cells. Genetic inactivation of this adaptor significantly reduces mammary breast cancer development and progression. Given the recognized importance of this adaptor in metabolic insulin resistance, our findings implicate Grb14 as a putative link between breast cancer and diabetes.

Nothing to Disclose: WL, LZ, SS, MG, SA, SE

Pub # OR06-5

Session Information ORAL SESSION: TRANSLATIONAL - Steroidal Regulation of Breast & Prostate Cancer (11:15 AM-12:45 PM)

Title GPR30: A Potential New Therapeutic Target for Castration-Resistant Prostate Cancer

Author String H-m Lam, B Ouyang, S-m Ho
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Body Prostate cancer (PCa) is the most common non-skin cancer in the U.S.. Despite the advances in treatments, nearly all PCAs which respond to hormone-ablation therapy turn into hormone-refractory cancer. These patients are left with very few treatment options and quickly succumb to the disease. G protein-coupled receptor 30 (GPR30) is a member of the G protein-coupled receptor superfamily, expressed at the cell membrane and/or at the endoplasmic reticulum. We recently demonstrated that activation of GPR30 through the use of a GPR30 agonist, G-1, inhibited the growth of PCa cell lines through activation of Erk/p21 pathways and induction of G2 arrest. These findings open a new door for using G-1 or other GPR30 agonists as therapies for PCa. However, the regulation of GPR30 in PCa is unclear and the therapeutic window of its activation is yet defined.

Hence, in this study, we determine if GPR30 is regulated by androgen and its potential to treat castration-resistant PCa. We demonstrated that androgen suppressed GPR30 expression in androgen receptor (AR)-positive LNCaP but not in AR-negative PC-3 and DU145 cells. Bicalutamide (an AR antagonist) and siRNA-AR abolished the inhibition, suggesting that AR mediates the androgen-induced downregulation of GPR30 expression. To investigate if the removal of androgen enhance the expression/action of GPR30, we established a castration-resistant LNCaP xenograft model which recapitulates hormone-refractory PCa growth after orchiectomy in human. In vivo results showed that GPR30 expression increased in xenografts re-emerged after castration. Most significantly, we found that G-1 did not inhibit growth nor induced significant necrosis in tumors grown in intact mice. However, G-1 was highly effective in halting the growth of the tumors that re-emerged after castration of their hosts. G-1 induced complete blockade of growth and a 66% necrosis in these castration-resistant tumors. These findings indicate that castration or removal of androgen can increase GPR30 expression and then sensitizes LNCaP tumors to G-1-induced cell growth inhibition via induction of necrosis.

In sum, we demonstrated that androgen inhibited GPR30 expression via AR and the removal of androgen sensitizes the PCa tumors to G-1-induced tumor necrosis and growth inhibition likely through elevating GPR30 expression. This study defines an effective window for GPR30 target therapy for castration-resistant PCa and it can be used following androgen-ablation therapy.

Sources of Research Support: US National Institutes of Health: ES006096, ES015584, CA015776, and CA112532.

Nothing to Disclose: H-ML, BO, S-MH

Pub # OR06-6
Session Information ORAL SESSION: TRANSLATIONAL - Steroidal Regulation of Breast & Prostate Cancer (11:15 AM-12:45 PM)
Title Loss of Caveolin-1 Increases Tumor Cell Migration, Is Predictive of Disease-Free Survival, and Induces Steroidogenesis in Prostate-Derived Fibroblasts
Author String M Morello, GE Ayala, F Rosati, G Danza, R Li, A Frolov, RM Adam, DR Rowley, TC Thompson, MP Lisanti, MR Freeman, D Di Vizio
Children's Hospital Boston, Boston, MA; Harvard Medical School, Boston, MA; Baylor College of Medicine, Houston, TX; University of Florence, Florence, Italy; Baylor College of Medicine, Houston, TX; Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA

Body Expression of stromal caveolin-1 (Cav-1), a membrane protein involved in signal transduction and cholesterol transport, is significantly lost in metastatic prostate cancer (PCa) (1). The role of Cav-1 in the stroma appears antithetical to its tumorigenic role in the prostate epithelium, however the underlying mechanisms are largely unknown. Recent evidence points to a role for intratumoral production of androgens in the transition from androgen responsive to castration-resistant PCa (CRPC), in which altered lipid metabolism and increased steroidogenesis have been documented (2). The role of the tumor stroma in producing newly synthesized androgens is unexplored. In this study, we show that loss of Cav-1 in prostate stroma-derived myofibroblasts results in gene expression alterations of modulators of adhesion, angiogenesis, invasion and metastasis. Tenascin C and the metalloprotease MMP1, markers of reactive stroma and of tumor cell dissemination, respectively, were dramatically upregulated. Cav-1-depleted myofibroblasts exhibited activation of cell signaling, increased cell proliferation and reduced apoptosis, while inducing cell migration in epithelial tumor cells. Decreased levels of stromal Cav-1, in a large prostate tissue microarray (>840 cases), directly correlated with Gleason score ($p < 0.001$) and inversely with recurrence-free survival ($p = 0.0385$). Gas chromatography-mass spectrometry (GC-MS) revealed a significant increase ($p < 0.001$) of intracellular cholesterol levels in Cav-1-depleted stromal cells, in comparison with the parental subline. RT-PCR revealed a significant increase in mRNA expression of enzymes involved in various steps of steroidogenesis. Steroidogenesis Acute Regulatory Protein (StAR), a key cholesterol transporter from the cytosol to the mitochondria was significantly up-regulated ($p = 0.011$) after Cav-1 silencing. A similar result was obtained for levels of CYP11A1 and HSD3B1, pivotal enzymes in the initial steps of steroidogenesis, and of 5α -reductase type 1 (SDR5A1), which catalyzes the conversion of circulating testosterone to dihydrotestosterone (DHT), and is upregulated in metastatic prostate cancer. These findings suggest that alterations in intracellular cholesterol levels and steroidogenesis in prostate-derived fibroblasts after Cav-1 silencing might play a role in establishing a 'permissive' tumor microenvironment for progression to CRPC disease.

(1) Di Vizio D. et al., Cell Cycle 2009; 15: 2420

(2) Locke et al., Cancer Res 2008; 68: 6407

Sources of Research Support: NIH Grant K99 CA131472 awarded to DDV; NIH Grant R01 DK57691 awarded to MRF; TMEN network awarded to GEA.

Nothing to Disclose: MM, GEA, FR, GD, RL, AF, RMA, DRR, TCT, MPL, MRF, DDV

Pub #	M4
Session Information	MEET-THE-PROFESSOR: CLINICAL - Laboratory & Radiologic Evaluation of the Adrenal Incidentaloma (11:15 AM - 12:00 PM)
Title	Laboratory & Radiologic Evaluation of the Adrenal Incidentaloma
Author String	AH Hamrahian Cleveland Clinic Foundation, Chagrin Falls, OH
Body	Nothing to Disclose: AHH

Pub # OR07-1

Session Information ORAL SESSION: CLINICAL - Diabetes: New Treatments & Exotic Cases (11:15 AM-12:45 PM)

Title Continuous, Subcutaneous IGF-I Therapy Via Insulin Pump in a Patient with Donohue Syndrome

Author String DE Stanescu, DR Weber, CJ Holland, SN Magge
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Body

Background: Donohue syndrome is the most severe end of a spectrum of syndromes of congenital insulin receptor (InsR) dysfunction, with a very limited life expectancy. Given the similarities in insulin and IGF1 receptors and signaling, IGF1 has been used to treat InsR mutation syndromes, and postulated to preserve β cell function. However, the shortened IGF1 half life due to low IGF-BP3 levels in these patients can be problematic.

Clinical Case: Patient was IUGR at birth, weighing 1.6 kg, with dysmorphic facies, decreased SC fat, polycystic ovaries, hyperglycemia, and insulin of 4925 uU/mL. InsR sequencing revealed an insertion in exon 2 causing an immediate stop codon (Y87X). Management goals were glycemic control and weight gain. Pt did not require treatment until 15mos, when she had diabetic ketosis. High-dose insulin suppressed ketones, but did not correct hyperglycemia. Metformin 30mg/kg/day was initiated. At 19 mos, HbA1c peaked at 9.5%. IGF1 SC BID was started at 80 mcg/kg/day, and increased for hyperglycemia to 400 mcg/kg/day. HbA1c improved over 6 mos to 7.7%. When BID dosing was no longer effective, continuous IGF1 via insulin pump was started at 31 mos. IGF1 dose was gradually increased to 1200 mcg/kg/day for hyperglycemia. IGF1 was maintained at 40-50 degrees F. HbA1c decreased from 9.8 to 8.8% over 3 mos.

Pre- and 1-hr post-feed c-peptide and insulin were measured to assess insulin resistance (IR) (fasting level) and β cell function ([Delta]change with feed). Prior to IGF1: fasting c-peptide (CPEP) = 37ng/ml, [Delta] = 69; fasting insulin (INS) = 1953uU/ml, [Delta] = 1172. On BID IGF1: After 7.5mos CPEP = 44ng/ml, [Delta] = 83, INS = 1238uU/ml, [Delta] = 2732; After 12mos CPEP = 25.8ng/ml, [Delta] = 58.9; INS = 3382uU/ml, [Delta] = 1677. After 1 mos on IGF1 pump: CPEP = 26.5ng/ml, [Delta] = 33.9.

Enlargement of cystic ovaries accelerated from 30 to 35 mos, when pt had a 9.5x15x11.4cm cystic abdominal mass, causing respiratory distress. Pathology showed juvenile granulosa cell tumor.

Conclusion: This is the first report of continuous SC IGF1 therapy via insulin pump in a pt with congenital InsR mutation, which may be advantageous given the decreased IGF1 half life. Glycemic control appeared improved, but effects on IR and β cell function are difficult to interpret. It is unclear whether ovarian growth and tumor were due to high-dose IGF1, or to the natural history of disease given pt's prolonged survival. Continuous IGF1 to treat patients with InsR mutations has the potential for benefit and warrants further study.

Disclosures: SNM: Advisory Group Member, Ipsen. Nothing to Disclose: DES, DRW, CJH

Pub # OR07-2

Session Information ORAL SESSION: CLINICAL - Diabetes: New Treatments & Exotic Cases (11:15 AM-12:45 PM)

Title Androgenic and Growth Factor Effects of Improved Insulin Sensitivity Following Leptin Therapy in Lipodystrophy

Author String AO Lungu, A Goodling, E Safar Zadeh, EK Cochran, P Gorden
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Body Background: The lipodystrophies (LD) are characterized by the deficiency of the adipocyte hormone leptin, resulting in metabolic abnormalities (insulin resistance, hypertriglyceridemia, and diabetes). Therapeutic administration of recombinant leptin improves insulin sensitivity, correcting these metabolic defects. We have asked whether other features of insulin resistance are corrected by increasing insulin sensitivity as a function of recombinant leptin treatment. Insulin-like growth factor-1 (IGF-1) is known to be depressed in insulin resistance, as well as sex hormone binding globulin (SHBG), whereas serum testosterone (T) is known to be increased in women. We have examined these parameters following recombinant leptin administration in LD patients.

Methods: 44 patients with LD enrolled in leptin replacement trial from 2000-present. Leptin was administered subcutaneously in doses 0.06-0.24 mg/kg/day and the different parameters were assessed at baseline, 4-8 months and 1 year of therapy.

Results: The mean triglyceride (TG) level at baseline of 878.3 ± 1250.9 mg/dL, decreased at 4-8 months (430.4 ± 688.2 mg/dL), and continued to decrease at the 1 year mark (306.5 ± 411.3 mg/dL). The mean hemoglobin A1C at baseline was 8.4% and decreased to 6.8% at 12 months. 34% patients were on insulin therapy at baseline and only 20% were on reduced doses of insulin after 12 months on leptin. The mean IGF-1 level at baseline was 155.1 ± 106.1 ng/mg, increased at 4-8 months (208.6 ± 149.9 ng/mL) and remained above the baseline level at 1 year (203.8 ± 118.3 ng/mL). 66% of patients had an increase in the IGF1 level at 1 year.

The mean SHBG level at baseline was 17.1 ± 17.5 nmol/L, increased at 4-8 months (29.7 ± 32.5 nmol/L), and remained above the baseline level at 1 year (26.7 ± 20.1 nmol/L). 83% of patients had an increase in SHBG after 1 year on leptin.

The mean free testosterone (FT) level at baseline was 2.93 ± 2.64 ng/dL. FT levels decreased at 4-8 (1.93 ± 1.34 ng/dL), and 1 year (1.69 ± 1.32 ng/dL). FT decreased in 65% of women at the 1 year mark.

Conclusion: In the present study, we show that both growth factors and other endocrine abnormalities that are common in Polycystic Ovarian syndrome, i.e. SHBG and FT, are also corrected by recombinant leptin administration in patients with LD. Thus both the metabolic and androgenic features of the syndrome are corrected as one moves from an insulin resistant to an insulin sensitive state.

Nothing to Disclose: AOL, AG, ESZ, EKC, PG

Pub # OR07-3

Session Information ORAL SESSION: CLINICAL - Diabetes: New Treatments & Exotic Cases (11:15 AM-12:45 PM)

Title Metreleptin for Treatment of Diabetes Mellitus and/or Hypertriglyceridemia Associated with Lipodystrophy: Translation from Clinical Trials to Clinical Endocrinology Practice

Author String SL Amarnath, S Kamineni, A Neidert, Y Kalagara, V Uhley, JL Chan, EA Oral
The University of Michigan, Ann Arbor, MI; Amylin, San Diego, CA

Body INTRODUCTION: Lipodystrophy (LD) syndromes are associated with severe metabolic derangements such as diabetes mellitus (DM) and hypertriglyceridemia (HT). Patients with LD and leptin deficiency who are treated with metreleptin (recombinant-methionyl human leptin) showed significant improvements in glycemic control and lipid levels over an extended period of time. We evaluated whether patients with a just a clinical diagnosis of partial LD (without confirmation of low leptin state) would benefit from metreleptin therapy. METHOD: From April 2009 to date, we enrolled 14 patients with various forms of partial LD (familial or acquired) into an open -label prospective study within the context of an expanded access program sponsored by Amylin Pharmaceuticals (San Diego, CA) to make metreleptin available to LD patients with DM and/or HT. Six of these patients (all women, age 29 to 63 years, BMI range: 21 to 39 kg/m²) have received metreleptin treatment for at least 9 months and up to 18 months at a dose of 0.04 to 0.12 mg/kg/day (2.2 to 12 mg) , which was titrated based on clinical response. RESULTS: We observed significant improvements in glycemic control (mean \pm SD HbA1c at baseline 8.7 \pm 1.4% vs. 6.8 \pm 0.4 post[minus]therapy, p=0.036) and a clinically meaningful trend for improvement in triglyceride levels (306 \pm 315 vs. 176 \pm 110 mg/dL, p=0.10) at 9 months. These improvements occurred in the setting of a reduction in insulin therapy in all with >50% reduction from baseline in 5 of 6 patients. Peak response was noted at 9 to 12 months of therapy with the dose titration employed. Metreleptin treatment was associated with a modest reduction in body weight (ranging from 3 to 7% from baseline in patients with BMI >27 kg/m²). Metreleptin doses up to 0.12 mg/kg/day (12 mg) were well-tolerated with nausea and injection site reactions being the most common adverse events. CONCLUSION: Metreleptin treatment in patients with partial LD selected just on the basis of simple clinical diagnosis (without confirmation of a low leptin state) results in significant improvements in HbA1c (generally in the setting of substantial reductions in insulin doses) and a trend towards improvement in triglyceride levels after 9 months of therapy.

Sources of Research Support: Amylin Pharmaceuticals.

Disclosures: JLC: Employee, Amylin Pharmaceuticals. EAO: Principal Investigator, Amylin Pharmaceuticals
Nothing to Disclose: SLA, SK, AN, YK, VU

Pub # OR07-4

Session Information ORAL SESSION: CLINICAL - Diabetes: New Treatments & Exotic Cases (11:15 AM-12:45 PM)

Title A Case of Berardinelli-Seip Congenital Lipodystrophy with a Novel Mutation, *BSCL2*/W107X, Presenting with Neonatal-Onset Diabetes

Author String RF Vasanwala, ZM Ng, L Ngee, F Yap
K K Women's and Children's Hospital, Singapore

Body **Background:** Berardinelli-Seip Congenital Lipodystrophy (BSCL) is a rare autosomal recessive condition characterised by generalised lack of body fat from birth. Patients with BSCL exhibit extreme hyperinsulinemia, hypertriglyceridemia and hepatomegaly during infancy, and develop diabetes mellitus mostly during pubertal years. However, neonatal-onset of diabetes mellitus in BSCL has not been reported previously.

Clinical case: A male infant was born to first-cousin Chinese parents at term gestation with a birth weight of 2670g. He had subtle facial dysmorphism, prominent skin folds and generalised lack of subcutaneous fat at birth. Other clinical features included hepatomegaly, lack of buccal and gluteal fat, protuberant abdomen and a lean but not muscular habitus.

On day 16 of life he developed vomiting and abdominal distension with high blood glucose (BG) of 24 mmol/l. He was empirically treated for presumed sepsis. Microbiological evidence of infection was not found. He then improved clinically but his hyperglycaemia persisted. He was investigated and started on insulin therapy, initially via intravenous infusion and subsequently by subcutaneous injections. He required insulin doses up to 2.8 units/kg/day with difficulty in normalising his BG.

His metabolic work-up showed low blood ketones (0.1mmol/l), raised C-peptide (12.1 mcg/L) and insulin (30.2 mU/L), and high total cholesterol (6.1mmol/l) and triglycerides (23.6 mmol/l. GAD and ICA autoantibodies were negative.

Given the clinical presentation of neonatal diabetes, insulin resistance, hypertriglyceridemia and generalised lipodystrophy, his blood was sent for molecular genetics analyses. He was found to be homozygous for a novel nonsense mutation, W107X, in exon 3 of the *BSCL2* gene. Both parents were found to be heterozygous carriers for the same mutation.

During subsequent follow up, his insulin requirement gradually declined and plasma triglycerides dropped. By 3 months, he no longer required exogenous insulin to maintain normoglycaemia. At 4 months, he weighed 4 kg and had reached appropriate developmental milestones.

Conclusion: This is the first report of BSCL presenting with neonatal-onset diabetes mellitus. Molecular testing was guided by clinical context and led to the identification of a novel *BSCL2* gene mutation in this kindred. This finding has important clinical implications in his management and genetic counselling for the family.

Nothing to Disclose: RFV, ZMN, LN, FY

Pub # OR07-5

Session Information ORAL SESSION: CLINICAL - Diabetes: New Treatments & Exotic Cases (11:15 AM-12:45 PM)

Title Liraglutide as Additional Treatment in Type 1 Diabetes

Author String A Varanasi, N Bellini, D Rawal, M Vora, A Makdissi, S Dhindsa, A Chaudhuri, P Dandona
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Body In view of the inability of insulin based treatments to control type 1 diabetes adequately in a consistent fashion and to avoid glycemic oscillations, we asked the question whether the addition of liraglutide to insulin treatment would improve the glycemic control since GLP-1 and its agonists are known to suppress post prandial increases in glucagon concentration in type 2 diabetics. Fourteen patients with well controlled type 1 diabetes (mean HbA1c: 6.5%) on continuous glucose monitoring and intensive insulin therapy on CSII were included in the study. Their glycemic control was optimized through careful regulation of their carbohydrate intake and insulin regimes for at least two weeks. No significant change in glycemic control or insulin dose was observed. Treatment with liraglutide was then started for either one week (n=14) or 24 weeks (n=14). Mean fasting glucose (130±10 to 110±8 mg/dl; p<0.05) and mean weekly glucose concentrations (138±20 to 115±12 mg/dl; p<0.05) fell significantly while the basal insulin (25±6 to 17±6 U/day; p<0.05) and bolus insulin (23±4 to 16±4 U/day; p<0.05) doses also fell significantly within 1 week. The oscillations in blood glucose concentrations were significantly reduced and the weekly CV for glucose concentrations fell from 39.6±10 to 22.6±7% (p<0.05). The patients who continued on liraglutide for 24 weeks maintained a similar glycemic control but they had further reductions in insulin doses and a significant weight loss (68.0±5 to 63.5±4 Kg; p<0.05). The mean HbA1c was reduced significantly from 6.5±0.5% to 6.1±0.4% (p<0.05). The withdrawal of liraglutide resulted in a rapid reversal of these effects and the restoration of marked glycemic oscillations. The addition of liraglutide to CSII results in a rapid and significant reduction in mean fasting and weekly glucose concentrations, reduction in glycemic oscillations and CV, simultaneously with a reduction in the dose of insulin in type 1 diabetics. In addition, there is a significant weight loss and a reduction in HbA1c.

Sources of Research Support: NIH Grants R01-DK075877 and R01-DK069805 awarded to PD; American diabetes Grant awarded to PD; American Diabetes Grant 10-JF-13 awarded to SD.

Disclosures: SD: Speaker, Abbott Laboratories. AC: Speaker, Eli Lilly & Company. PD: Principal Investigator, GlaxoSmithKline; Clinical Researcher, Sanofi-Aventis; Speaker, Novartis Pharmaceuticals; Eli Lilly & Company; GlaxoSmithKline; Sanofi-Aventis. Nothing to Disclose: AV, NB, DR, MV, AM

Pub # OR07-6

Session Information ORAL SESSION: CLINICAL - Diabetes: New Treatments & Exotic Cases (11:15 AM-12:45 PM)

Title Reduced Number of Transplantations with Equivalent Long-Term Glycemic Control in Single Initial Islet Transplantation as Compared to Multiple Initial Islet Transplantation

Author String E Ajdler-Schaeffler, PA Gerber, RA Zuellig, T Pfammatter, GA Spinas, R Lehmann
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Body The main goal of islet transplantation in patients with type 1 diabetes mellitus has changed over the last years from insulin independence to good glycemic control and avoidance of severe hypoglycaemia. No data exists about the optimal initial islet mass. In this study we compared patients who underwent combined islet-kidney or islet after kidney transplantation at our institution with multiple initial islet transplantations (11 patients with maximal 5 transplants) as compared to single initial islet transplantation (22 patients who were retransplanted only if good metabolic control was no longer achieved). The time between the first and second transplantation was 2.4 ± 2 and 25.8 ± 9 months, respectively. There was no significant difference in baseline characteristics in the two groups: Age was 50.8 ± 8.5 and 53.6 ± 7.4 y with 45.5% and 72.7% male patients, diabetes duration of 38.1 ± 7.8 and 37.4 ± 11.3 y. Total follow-up (FU) was 78 and 51 months. Islet retransplantation was performed 0.35 times per patient-year of FU in the 1st group and 0.11 times in the 2nd group. HbA1c decreased significantly in both groups after transplantation ($p=0.017$ and $p=0.026$) but did not differ between the groups during FU (Figure 1). Frequency of severe hypoglycaemia was comparable in the two groups after transplantation (0.16 vs 0.13 hypoglycaemia/patient-year, $p=0.88$) and occurred only in 15.1% of all patients. Insulin dosage decreased significantly in the 1st group (from 0.62 IE/kg to 0.25 IE/kg, $p=0.017$) during the first year after transplantation, but not in the 2nd group (from 0.54 IE/kg to 0.4 IE/kg, $p=0.38$). Similarly, C-peptide was higher one year after transplantation in the 1st group (1454 ± 554 pM) as compared to 655 ± 443 pM ($p=0.028$) in the 2nd group (single transplants). This study demonstrates that a single initial transplantation with retransplantation only if glycemic control deteriorates, uses less pancreas donors as compared to multiple initial transplantations, but results in equal glycemic control and the same rate of severe hypoglycemic episodes.

Nothing to Disclose: EA-S, PAG, RAZ, TP, GAS, RL

Pub #	M5
Session Information	MEET-THE-PROFESSOR: CLINICAL - Management of Hypertriglyceridemia (11:15 AM - 12:00 PM)
Title	Management of Hypertriglyceridemia
Author String	J Brunzell University of Washington, Seattle, WA
Body	Nothing to Disclose: JB

Pub # OR08-1

Session Information ORAL SESSION: CLINICAL - Thyroid Cancer (11:15 AM-12:45 PM)

Title Use of Radioactive Iodine for Thyroid Cancer in the United States, 1990-2008

Author String MR Haymart, M Banerjee, AK Stewart, RJ Koenig, JD Birkmeyer, JJ Griggs
University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI; Commission on Cancer, Chicago, IL

Body **Background:** Substantial uncertainty persists over the indications for radioactive iodine after total thyroidectomy. Use of radioactive iodine over time and the correlates of its use remain unknown. **Methods:** We performed time trend analysis of radioactive iodine use in a cohort of 189,219 well-differentiated thyroid cancer patients treated at 981 hospitals associated with the National Cancer Database between 1990-2008. We then used multilevel analysis to assess influence of patient, tumor, and hospital characteristics on radioactive iodine use in the cohort treated from 2004-2008. **Results:** Between 1990 and 2008, across all tumor sizes, there has been a significant rise in the proportion of well-differentiated thyroid cancer patients receiving radioactive iodine ($P<0.001$). Multivariable analysis of patients treated from 2004 to 2008 found that, in addition to tumor and patient characteristics, hospital volume and region were associated with a greater likelihood of radioactive iodine use. Wide variation in radioactive iodine use existed and only 24.5% of this variation was accounted for by tumor characteristics and 17.9% by patient characteristics. Hospital type, case volume, and region, together accounted for 17.4% of the variation. After adjusting for all available patient, tumor, and hospital characteristics, much of the variance, 29.6%, was attributable to unexplained hospital characteristics. **Conclusions:** The proportion of well-differentiated thyroid cancer patients treated with radioactive iodine has increased over time. There is tremendous between-hospital variation in radioactive iodine use and the hospital where care is received may have a greater influence on receipt of radioactive iodine than patient or tumor characteristics.

Nothing to Disclose: MRH, MB, AKS, RJK, JDB, JJG

Pub # OR08-2

Session Information ORAL SESSION: CLINICAL - Thyroid Cancer (11:15 AM-12:45 PM)

Title Low-Risk Differentiated Thyroid Cancer with BRAFV600E Mutation Is More Difficult to Cure Than Negative Cases: A 5-Year Follow-up Study

Author String D Viola, R Giannini, C Ugolini, A Biagini, C Romei, E Molinaro, L Agate, F Basolo, A Pinchera, R Elisei
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Body BRAFV600E mutation is the most frequent genetic alteration (29-83%) in the primary tissue of papillary thyroid carcinoma (PTC). Many authors have demonstrated that the presence of the mutation is associated with a more advanced tumor stage at diagnosis and a worse outcome but anyone assessed if BRAFV600E mutation could be useful to evaluate the prognosis of low risk patients (STAGE I according to the 7th TNM classification).
Aim of this study was to evaluate if the presence of BRAFV600E mutation in the primary tumor could be a predictor of persistent disease in low risk patients (Stage I).
We retrospectively analyzed the clinicopathological features of 156 STAGE I PTC patients treated with total/near total thyroidectomy and iodine-131. Genomic DNA was purified from paraffin-embedded tumoral tissue. A PCR SSCP analysis of exon 15 of BRAF was performed and direct sequencing of SSCP positive cases was made.
The mutation was present in 60 patients (38.5%) and was significantly associated to the absence or invasion o tumoral capsule ($p=0.001$), aggressive histological variant (0.003) and extrathyroid extension ($p=0.04$). After 5 years of follow up, 23 (14.7%) patients had persistent disease and 133 (85.3%) were free of disease. BRAFV600E mutation was present in 45 patients (45/133= 33.8%) free of disease and in 15 (15/23= 65.2%) patients with persistent disease with a statistically significant difference ($p=0.004$). At univariate analysis persistent disease was associated also to tumor size ($p=0.02$), the presence of BRAFV600E ($p=0.004$) extrathyroid extension ($p=0.01$) and node metastases ($p<0.0001$). However at multivariate analysis only the presence of BRAFV600E mutation significantly correlated with persistent disease ($p=0.005$).
In conclusion, our results show the correlation of BRAFV600E mutation with aggressive features also in low risk PTC and indicate that the presence of BRAFV600E mutation is a bad prognostic factor for the persistence of disease also in low risk PTC.

Nothing to Disclose: DV, RG, CU, AB, CR, EM, LA, FB, AP, RE

Pub # OR08-3

Session Information ORAL SESSION: CLINICAL - Thyroid Cancer (11:15 AM-12:45 PM)

Title Cost-Effectiveness of a Novel Molecular Test for Cytologically Indeterminate Thyroid Nodules

Author String H Li, KA Robinson, B Anton, IJ Saldanha, PW Ladenson
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Johns Hopkins Medical Institutions, Baltimore, MD

Body

Background: Determining which patients with thyroid nodules require surgery is limited by cytologically indeterminate findings. A novel approach was recently described for molecular classification of cytologically indeterminate thyroid nodules with a sensitivity of 91% and specificity of 75%. To assess the impact on costs and patient quality-of-life from a societal perspective, we performed a cost-effectiveness analysis comparing this novel test to current practice for cytologically indeterminate nodules.

Methods: Our 16-state Markov decision model was based on ATA guidelines. Direct medical costs and effectiveness in quality-adjusted life years (QALYs) were estimated based on literature review, DHHS data, Medicare reimbursement, and expert opinion. The decision analysis and subsequent probabilistic sensitivity analyses were performed using Monte Carlo simulation.

Results: Modifying current practice with use of the novel molecular test resulted in 74% fewer surgeries for benign nodules with no greater number of untreated cancers. Over the 5 years after an indeterminate biopsy, mean discounted cost estimates (initial testing, surgery, follow-up, managing complications, and treating recurrence) were \$11,491 for current practice and \$9,853 with the molecular test. Current practice and molecular test use produced 4.50 and 4.56 QALYs, respectively. Sensitivity analyses revealed that model factors with greatest impact were test specificity and cost, and probabilities related to surgical decision-making. However, probabilistic sensitivity analyses demonstrated that both the cost savings and health benefits persisted over a wide range of randomly sampled likely parameter values. In 98.5% of 10,000 simulations, use of the molecular test offered both cost savings and health benefits; none of the simulations showed the molecular test would be either more costly and less effective, or less costly and less effective.

Conclusions: This health economic analysis demonstrated that use of a novel molecular test for differential diagnosis of cytologically indeterminate thyroid nodules avoided almost three-fourths of currently performed surgeries in patients proving to have benign nodules. From a societal perspective, use of this test would result in lesser overall costs and modestly improved quality-of-life for patients than current practice based on cytological findings alone.

Sources of Research Support: Veracyte, Inc.

Disclosures: PWL: Consultant, Veracyte, Inc. Nothing to Disclose: HL, KAR, BA, IJS

Pub # OR08-4

Session Information ORAL SESSION: CLINICAL - Thyroid Cancer (11:15 AM-12:45 PM)

Title Circulating TSHR mRNA in Preoperative Detection of Thyroid Cancer in Patients with Thyroid Nodules: Diagnostic Performance According to Final Histopathology of Cancer

Author String M Gupta, M Milas, J Brainard, J Shin, C Nasr
Cleveland Clinic, Cleveland, OH; Cleveland Clinic, Cleveland, OH; Cleveland Clinic, Cleveland, OH; Cleveland Clinic, Cleveland, OH

Body **Introduction:** TSHR-mRNA a marker for circulating thyroid cells is detected using quantitative RT- RT-PCR assay [JCEM 2007; 92:468-475]. This test is routinely performed in our lab for patients seen for thyroid nodules.

Materials and methods: During 2009 a total of 348 patients with thyroid nodules who had total thyroidectomy and final pathologic diagnosis were retrospectively reviewed. This data was analyzed by ROC analysis to assess its performance for the preoperative diagnosis of thyroid cancer in relation to histopathology of cancer. Diagnostic sensitivity, specificity and overall efficacy for preoperative detection of papillary thyroid cancer[PTC], PTC microcancers and follicular cancers was determined.

Results: Out of 348 patients 194 had thyroid cancer and 154 had benign disease [74 had FNA biopsy]. Among thyroid cancer patients 95 [84 had FNA biopsy] had papillary thyroid cancer [PTC>1cm], 78 had micro PTC, 18 had Follicular cancer [FC] and 5 had other pathologies. ROC analysis showed maximum diagnostic efficiency at cut off level of 0.7 ng /[micro]g of total RNA. Out of 154 benign cases 29 were positive [specificity 80.5%]. The diagnostic sensitivity and efficiency for all patients was 67% and 73%, for PTC was 82% and 81%; for Micro-PTC was 53% and 71% and for follicular cancers [FC] was 44% and 60%. The test showed the highest efficiency for detection of PTC >1.0 cm.[PPV of 72% and NPVof 88%]. Among 84 PTC patients who had FNA biopsy, FNA was indeterminate in 33[39%] and was negative in 7[8%] patients. Among 74 patients with FNA biopsy and benign disease, FNA was negative in 29[39%] and was indeterminate in 45 [61%] [15 atypical/suspicious and 30 follicular lesions]. TSHR mRNA correctly classified 61 of 78 [78%] patients with indeterminate biopsy results [30/43 atypical and 31/35 follicular lesions] and 5/7 cancer patients with benign FNA. The diagnostic sensitivity for PTC in patients with FNA was 79% and specificity was 88%. It has PPV 88%, NPV 78% and overall accuracy of 83%.

Conclusion: Among thyroid cancers TSHR mRNA demonstrates high sensitivity and specificity for the detection of PTC preoperatively and its primary value resides in the diagnosis of cancer among patients with indeterminate FNA.

Nothing to Disclose: MG, MM, JB, JS, CN

Pub # OR08-5

Session Information ORAL SESSION: CLINICAL - Thyroid Cancer (11:15 AM-12:45 PM)

Title The Inheritance of *CYP1A1m1* and *CYP1A2* Polymorphisms May Increase the Susceptibility to Medullary Thyroid Carcinoma

Author String RB Barbieri, NE Bufalo, ACdN Da Silva, LVM Assumpcao, FF Valente, RMdB Maciel, JM Cerutti, LS War State University of Campinas, Campinas, Brazil; Federal University of São Paulo, São Paulo, Brazil

Body There is considerable variability in the clinical presentation and evolution of medullary thyroid carcinoma (MTC) patients, even when they have identical *RET* gene. Besides *RET* polymorphisms, low penetrance gene: involved in the biotransformation of environmental xenobiotics could explain such variability. In fact, we and others had demonstrated that the inheritance of a particular genetic profile for metabolizing enzymes, may modify the risk of development of differentiated thyroid cancer. In order to evaluate the influence of these inheritance on MTC risk and patients' outcome, we used TaqMan SNP to genotype for *CYP1A1m1* and *CYP1A2* 138 familial MTC (FMTC) patients; 49 sporadic MTC (SMTC); 89 FMTC probands' relatives and 578 control individuals matched on the basis of gender, age, ethnicity and previously sequenced for *RET* gene *CYP1A1m1* genotype profile was similar in FMTC (TT=77.3%;CT=20%; CC=2.6%) and in the control population (TT=68.2%;CT=29%; CC=2,8%), but the presence of a *CYP1A1m1* C allele was higher in SMTC (TT=54.3%;TC+CC=45.7%) than in controls (TT=68.2%;TC+CC=31.8%;p=0.023), increasing the risk for SMTC more than 2x (OR=2.45;95%CI=1.13-5.33). *CYP1A2* genotype profile was similar in SMTC patients and controls but the presence of C allele was over-represented in FMTC patients (AA=44%;AC+CC=56%) compared to controls (AA=47.8%; AC+CC=52.2%) increasing the risk of developing FMTC more than 2x (OR=2.10; 95% CI=1.11-3.97; p=0.022). The concomitant inheritance of the homozygous C allele in both *CYP1A1m1* and *CYP1A2* genes also increased the risk for FMTC (OR=2.34;95%CI=1.16-4.69;p=0.017), but did not increase the risk for SMTC. The analysis showed that the inheritance of a *CYP1A2* or *CYP1A1m1* gene C allele in homozygosis was more frequent in patients' relatives than in controls (80.8% vs 19% and 2.7% vs 7%, respectively) increasing the risk of FMTC (OR=2.40;95%CI=1.19-4.86;p=0.015 and OR=2.79;95%CI=1.04-7.51;p=0.042, respectively). We were unable to demonstrate any relationship between *CYP* and *RET* genotypes and between *CYP* and any clinical or pathological features of aggressiveness or outcome.

Our data indicate that the presence of C allele in *CYP1A1m1* and *CYP1A2* genes is a risk factor for the development of FCMT and SCMT, respectively. Relatives of probands with *CYP1A2* or *CYP1A1m1* gene C allele in homozygosis should be considered a group of risk hence deserving a more careful observation.

Sources of Research Support: FAPESP 2010/07067-9; CAPES.

Nothing to Disclose: RBB, NEB, ACdNDS, LVMA, FFV, RMdB, JMC, LSW

Pub # OR08-6

Session Information ORAL SESSION: CLINICAL - Thyroid Cancer (11:15 AM-12:45 PM)

Title Preparation with Recombinant Human Thyrotropin (rhTSH) Stimulation or Levothyroxine (T4) Withdrawal for Radioiodine (RAI) Treatment Gives Comparable Results in Patients with Metastatic Differentiated Thyroid Cancer (DTC)

Author String J Klubo-Gwiedzinska, KD Burman, D Van Nostrand, M Mete, L Wartofsky
Washington Hospital Center, Washington, DC; Washington Hospital Center, Washington, DC; MedStar Health Research Institute, Hyattsville, MD

Body **Background:** RAI therapy for DTC requires TSH elevation. For initial remnant ablation, there are two possible methods of TSH stimulation: (1) L-T4 withdrawal (THW) to provoke endogenous TSH elevation or (2) exogenous stimulation by rhTSH. Use of rhTSH for therapy of metastatic disease is not FDA approved except on a compassionate basis or when T4 release from metastases precludes endogenous TSH elevation. **Aim:** To compare the relative efficacy and side effect profile of rhTSH vs. THW for RAI therapy of metastatic DTC. **Methods:** Patients with RAI-avid distant metastases of DTC treated with RAI between 1996 and 2009 with either exclusively THW or rhTSH were retrospectively analyzed. Of 56 patients (31 women, 25 men) followed for 72+/-193 months (mean +/-SD) 15 patients had received 1-2 RAI therapies after rhTSH preparation, and 41 patients underwent 1-4 therapies after THW. The groups were comparable in regard to mean size of target lesions (rhTSH vs THW 6.4 vs 4.8 cm, p=0.41), mean baseline thyroglobulin (Tg) level (6995 vs 5544 ng/ml, p=0.83), distribution of micro-nodular pulmonary metastases (67% vs 63%, p=0.54), macro-nodular pulmonary metastases (13% vs 15% p=0.64), osseous metastases (53% vs 29%, p=0.09), brain metastases (0% vs 2%, p=0.73), and liver/kidney metastases (13% vs 2%, p=0.61). Patients undergoing rhTSH-aided RAI were older than those treated after THW (mean 62 vs 49 years, p= 0.01) and received significantly lower cumulative RAI dose (256 vs 416 mCi, p=0.03), which was more frequently based on dosimetric calculations (80% vs 46%, p=0.024). Response to treatment was based on RECIST 1.1 criteria. **Results:** Rates of complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) were not different between the groups (rhTSH vs THW CR 7% vs 12%, p=0.48, PR 0% vs 0%, p=n/a, SD 73% vs 56%, p=0.20, PD 20% vs 32%, p=0.31). Tg decreased after treatment in 79% of patients treated with rhTSH-aided RAI and 70% with THW-aided RAI (p=0.42). Progression free survival was not different between the groups (rhTSH vs THW 20 vs 24 months, p=0.55). Rates of leukopenia, thrombocytopenia, xerostomia and restrictive pulmonary disease were not significantly different (rhTSH vs THW 30% vs 28%, p=0.61, 10% vs 0%, p=0.37, 0% vs 12%, p=0.20, 0% vs 2%, p=0.73, respectively). **Conclusions:** Patients with metastatic DTC prepared with rhTSH achieve comparable benefit of RAI therapy and similar side effects as those treated after T4 withdrawal.

Nothing to Disclose: JK-G, KDB, DVN, MM, LW

Pub # OR09-1

Session Information ORAL SESSION: CLINICAL - Will Medical Management Replace Surgery for Cushing Syndrome? (11:15 AM-12:45 PM)

Title Surgical Management of Cushing Disease: The Birmingham, UK, Experience 1988-2009

Author String ZK Hassan-Smith, AP Johnson, W Arlt, A Toogood, MS Cooper, M Sherlock, PM Stewart
University of Birmingham, Birmingham, UK; University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK

Body

Introduction: Our aim was to meet the need for an up to date analysis of outcomes and mortality in Cushing's Disease (CD), using a cohort of patients treated over the past 2 decades.

Patients and Methods: We conducted a retrospective analysis of 80 patients who underwent trans-sphenoidal surgery to treat CD between 1988 and 2009. All operations were performed by a single surgeon, in a UK tertiary referral centre. In 72 patients data on clinical features, outcomes and mortality were collected from medical records. In 8 patients, records were unavailable, so mortality data was obtained using an NHS database.

Results: The median age at diagnosis was 40 years (IQR 31-50 years). The male to female ratio was 3.7. Median clinical follow up was 4.6 years (IQR 1.7-9.7 years). Median follow up for mortality was 10.9 years (IQR 4.9-15.6 years). Follow up data were available on 68/72 patients. 82%(56/68) achieved an initial disease remission, of which 8 suffered disease recurrence. Median time to recurrence was 2.1 years (IQR 1.3-3.1 years). 3 outcome groups were identified: 'Long-term remission' 70% (48/68), 'Persistent disease' 18%(12/68) and 'Recurrent disease' 12%(8/68). Long-term remission rates were significantly higher(82%) in patients with post-op cortisol levels <50nmol/L, compared to those with cortisols >50nmol/L (50%)(p<0.05). 82% of those with ACTH positive histology achieved long-term remission during follow up, compared to 58% with negative histology(p<0.05). All 14 patients treated with Endoscopic TSS achieved long-term remission. Further treatment for active disease included revision TSS(N=17), bilateral adrenalectomy(N=9), and radiotherapy(N=8). Common post-op complications included transient DI(37%), and CSF leak(15%). There was no statistically significant difference in complication rates between the endoscopic and microscopic approaches. 81% of patients had hypopituitarism of at least 1 pituitary hormone, with ACTH the most commonly affected. There were a total of 12 deaths in the group of 80. 4 deaths in the 'Long term remission' group, 2 in the 'Disease Recurrence' group, 1 with 'Persistent disease' and 5 with no clinical follow up data available. There was no significant difference in mortality between the groups.

Conclusions: Our favourable 'Long-term Remission' rates serve to underline the importance of an experienced surgeon the management of CD. Undetectable post-op cortisols and ACTH positive histology are favourable prognostic factors.

Disclosures: WA: Consultant, Roche Pharmaceuticals. Nothing to Disclose: ZKH-S, APJ, AT, MSC, MS, PMS

Pub # OR09-2

Session Information ORAL SESSION: CLINICAL - Will Medical Management Replace Surgery for Cushing Syndrome? (11:15 AM-12:45 PM)

Title Prediction of Postoperative Remission in Cushing Disease: Emory University Experience

Author String R Shah, NM Oyesiku, AG Ioachimescu
Emory University, Atlanta, GA; Emory University, Atlanta, GA

Body
Introduction: Possible predictors of postoperative remission in Cushing disease include: microadenoma by MRI, ACTH+ adenoma by pathology and low serum/urine cortisol during first postoperative days (POD). Previous studies yielded controversial results.
Methods: We reviewed all cases of Cushing's disease operated by 1 neurosurgeon between 12/1996-1/2010 with >2 months follow-up (N=49). Morning serum cortisol levels were collected on POD2-7 in 46 cases; 3 received steroids due to symptoms of adrenal insufficiency. Empirical steroids were not administered perioperatively. Early remission was defined as low or normal cortisol testing at 2-6 months postoperatively. Recurrence was defined by return of hypercortisolemia after a period of postoperative remission.
Results: The 49 patients (age 42.9±13.1, 10 M/39 F) were followed for a median of 16 months (2-110). Imaging showed microadenoma in 26, macroadenoma in 13 and no adenoma in 10 patients. POD2-7 nadir serum cortisol level was [le] 5 mcg/dL in 32 patients; of them, 20 had cortisol < 2 mcg/dL. Four patients with microadenoma had reoperation within 2 months; all had nadir cortisol >5 mcg/dL. Remission rate at 2-6 months was 91.8% (96.2% microadenoma, 92.3% macroadenoma and 80% in no-adenoma group), which included 3/4 patients with repeated surgery. All patients with nadir serum cortisol [le] 5 mcg/dl achieved early remission.
Twenty-eight patients were followed >10 months. Recurrence occurred in 6 cases at a median 32.7 months postoperatively (13.7-59.1). Of them, 2 had serum cortisol [le] 5 mcg/dL (1.8; 4 and 5 mcg/dl). In the group of 6 patients with recurrence, 5 had microadenomas and 1 normal preoperative MRI, and pathology identified the adenoma in all cases.
Statistical analysis showed that nadir serum cortisol [le] 5 mcg/dL on POD2-7 strongly predicted early (p<0.01), but not long-term remission (p=0.4). The identification of ACTH+ adenoma by pathology was borderline predictive of early (p=0.07), but not long-term remission. Age, gender, preoperative MRI and POD2-7 UFC did not predict early or sustained remission.
Conclusion: Surgery for Cushing disease at our institution yielded an early remission rate of 91.8%. All patients with nadir POD2-7 serum cortisol [le] 5 mcg/dL achieved early remission, but no level predicted lack of recurrence. UFC on POD2-7 and preoperative imaging did not yield additional prognostic information. Further research is needed to understand risk factors for late recurrence in Cushing's disease.

Nothing to Disclose: RS, NMO, AGI

Pub # OR09-3
Session Information ORAL SESSION: CLINICAL - Will Medical Management Replace Surgery for Cushing Syndrome? (11:15 AM-12:45 PM)
Title Prolonged Remission after Long-Term Treatment with Steroidogenesis Inhibitors in Ectopic Cushing Syndrome
Author String ST Sharma, LK Nieman
Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD

Body **Background:** Spontaneous remission occurs rarely in ectopic ACTH syndrome (EAS) (1-3). We describe three cases with EAS where long-term treatment with steroidogenesis inhibitors was followed by prolonged remission of hypercortisolemia. None of the patients had a history of cyclic hypercortisolism before referral.
Case Series:
Case 1: 56 year old woman diagnosed with EAS in 2002 with unsuccessful localization. Adrenal hyperplasia was seen on CT scan. To control hypercortisolism, she was maintained on ketoconazole (KTZ), metyrapone (MET) and dexamethasone (DEX) 0.5 mg/day. In 2006, KTZ and MET were discontinued due to an AM cortisol of <1.0 mcg/dl [normal (N): 5-25] and ACTH 7 pg/ml (N < 46), urine free cortisol (UFC) <0.52 mcg/24h (N: 3.5-45), and atrophic adrenal glands on CT. In 2007, AM cortisol of 6.1 mcg/dl (N: 5-25), ACTH 19 pg/ml (N <46), and a post-ACTH stimulation cortisol of 19 mcg/dl led to the discontinuation of DEX. Repeat imaging showed normal adrenal glands. She remains off DEX with no evidence of recurrent Cushing's syndrome (CS).
Case 2: 47 year old man diagnosed with EAS in 2002. Imaging studies showed a lung nodule but hypercortisolemia persisted after resection. He was maintained on KTZ but self-discontinued it in 1/2009. In 3/2009, AM cortisol level was 9.9 mcg/dl (N: 5-25), ACTH 44 pg/ml (N <46), and UFC 27 mcg/24h (N: 3.5-45). Post-ACTH stimulation, cortisol was 26.2 mcg/dl. He remains off all medications with no evidence of recurrent CS.
Case 3: 35 year old man diagnosed with EAS in 1999 with negative localization and bilateral adrenal hyperplasia on CT scan. KTZ was initiated in 1999; MET and Mitotane ([le] 2g daily) were added later to control hypercortisolemia. KTZ was discontinued in 3/2009 due to liver dysfunction. In 6/2009, he developed signs and symptoms of adrenal insufficiency. AM cortisol was 4 mcg/dl (N: 5-25), ACTH 242 pg/ml (N <46), and UFC 24 mcg/24h (N: 3.5-45). CT scan showed bilateral adrenal atrophy. Mitotane and MET were discontinued. Hydrocortisone replacement therapy was started and continued until 10/2010 when cortisol levels were found to be increased. Repeat testing was consistent with relapse of EAS with unsuccessful localization. Bilateral adrenalectomy was performed for definitive treatment of CS.
Conclusion:
Spontaneous and prolonged remission of hypercortisolemia can be seen in EAS after long-term treatment with steroidogenesis inhibitors; the mechanism by which this occurs is not clear.

1. Loh KC, Gupta R, Shlossberg AH. Spontaneous remission of ectopic Cushing's syndrome due to pheochromocytoma. *Eur J Endocrinol* 1996;135(4):440-3
2. Beardwell CG, Adamson AR, Shalet SM. Prolonged remission in florid Cushing's syndrome following Metyrapone treatment. *Clin Endocrinol* 1981;14:485-492
3. Silva F, Vazquez-Selles J, Aguilo F, et al. Recurrent ectopic adrenocorticotrophic hormone producing thymic carcinoid detected with octreotide imaging. *Clin Nuc Med* 1999;24(2):109-110

Sources of Research Support: Intramural program of the National Institute of Child Health and Human Development.

Nothing to Disclose: STS, LKN

Pub # OR09-4

Session Information ORAL SESSION: CLINICAL - Will Medical Management Replace Surgery for Cushing Syndrome? (11:15 AM-12:45 PM)

Title Results of 1,Ortho-1,para[prime]-Dichloro-Diphenyl-Dichloroethane (O,p[prime]DDD) Treatment in 76 Patients with Cushing Disease

Author String C Baudry, J Coste, R Bou Khalil, L Guignat, H Abbas, J Guibourdenche, S Silvera, X Bertagna, J Bertherat H[ocirc]pital Cochin, Assistance Publique-H[ocirc]pitaux de Paris, Paris, France; H[ocirc]pital Cochin, Assistance Publique-H[ocirc]pitaux de Paris, Paris, France; H[ocirc]pital Cochin, Assistance Publique-H[ocirc]pitaux de Paris, Paris, France; H[ocirc]pital Cochin, Assistance Publique-H[ocirc]pitaux de Paris, Paris, France

Body
Introduction : Alternative to transsphenoidal pituitary surgery (TSS) may be required in Cushing's disease (CD), as first or second line treatment. 1,ortho-1,para'-Dichloro-Diphenyl-Dichloroethane (o,p'DDD) has a potent anticortisolic action. Its place in CD treatment is not well defined. The aim of this study was to further evaluate the efficacy and tolerance of o,p'DDD in CD.
Patients and Methods: 76 patients treated with o,p'DDD out of a single center cohort of 219 patients with CD diagnosed between 1993 and 2009 were retrospectively studied. Remission was defined as normalization of 24 hours- urinary cortisol (24h-UC). Remission, recurrence and time-to-event were estimated by the Kaplan-Meier method, and potential predictors analyzed using Cox's proportional hazards regression models.
Results: Remission was achieved in 48 (72%) patients, with a median time of 6.7 months (5.2-8.2, 95% confidence limits). Plasma o,p'DDD (mean± SD) at the time of remission was 10.5 [μ]g/ml ± 8.9, with a mean daily dose of 2.6 ± 1.1 g. A negative linear relationship was observed between plasma o,p'DDD and 24h-UC (p<0.0001). Intolerance leading to treatment discontinuation occurred in 19 patients (29%). Recurrence after drug cessation occurred in 71% of patients, with a median time of 13.2 months (5.0-67.9, 95% confidence limits). Only high ACTH plasma level at the time of treatment discontinuation was statistically associated with a lower recurrence probability (HR 0.57 [0.32-1.00], p=0.05). A pituitary adenoma became visible during o,p'DDD treatment in 12 patients (25%) with initial negative pituitary imaging allowing subsequent TSS.
Conclusion : O,p'DDD is useful at different steps of CD management, either as first line when pituitary adenoma is not visible or because of the severity of hypercortisolism, or as a second line after TSS failure or recurrence, with most often easily manageable side effects. Monitoring of plasma o,p'DDD allows dosage adaptation to optimize hormonal control together with drug tolerance.

Disclosures: JB: Investigator, HRA Pharma. Nothing to Disclose: CB, JC, RBK, LG, HA, JG, SS, XB

Pub # OR09-5

Session Information ORAL SESSION: CLINICAL - Will Medical Management Replace Surgery for Cushing Syndrome? (11:15 AM-12:45 PM)

Title Mifepristone, a Glucocorticoid Receptor Antagonist, Produces Clinical and Metabolic Benefits in Patients with Refractory Cushing Syndrome: Results from the Study of the Efficacy and Safety of Mifepristone in the Treatment of Endogenous Cushing Syndrome (SEISMIC)

Author String M Fliseriu, BMK Biller, JW Findling, ME Molitch, DE Scheingart, C Gross
Oregon Health & Science University, Portland, OR; Massachusetts General Hospital, Boston, MA; Medical College of Wisconsin, Milwaukee, WI; Northwestern University Feinberg Medical School, Chicago, IL; University of Michigan, Ann Arbor, MI; Corcept Therapeutics, Menlo Park, CA

Body Mifepristone (MIFE), a glucocorticoid receptor (GR) antagonist, was studied (300-1200 mg po daily) in a 24-week multi-center, open-label trial in 50 subjects with Cushing's syndrome (Cushing's disease [N=43], ectopic ACTH [N=4], adrenal carcinoma [N=3]) who had failed multi-modal standard therapy. Two groups were enrolled based on predefined baseline glucose intolerance (DM/IGT) and/or hypertension (HT): C-DM (N=29) and C-HT (w/o DM/IGT, N=21). The primary analysis group was a modified intent to treat population (subjects receiving [ge]30 days of MIFE, mITT) composed of 25 C-DM, 21 C-HT (32 W, 14 M mean (\pm SD), age 44.5y [26-71], wt 99.5 \pm 29.6 kg, BMI 35.7 \pm 9.9. Response was predefined as a 25% reduction in AUCglucose on oGTT (C-DM) or 5 mmHg reduction in diastolic BP (DBP, C-HT) at final visit; statistical significance was met if the lower bound of the 95% CI for group response rates exceeded 20%. In C-DM an AUCgluc response was seen in 60% of subjects (95% CI lower bound 42%). In all mITT C-DM, mean (\pm SD) HbA1c decreased from 7.4 \pm 1.5% to 6.4 \pm 1.2% (P<0.001); FPG decreased from 149 \pm 75 to 110 \pm 38 mg/dL (P<0.02). In C-HT, a DBP response was seen in 38% of subjects (95% CI lower bound 21%). Mean change in weight was -5.7 \pm 7.4% with associated decreases in waist circumference. Insulin resistance, Cushingoid appearance, depression (Beck Depression Index), cognition (Trail A&B), and QoL (SF36) also improved. As expected with GR antagonism, increases were seen in mean ACTH (70[rarr]138 pg/mL), UFC (366[rarr]694 [micro]g/24h), am serum cortisol (23.8[rarr]35.8 [micro]g/dL), and late night salivary cortisol (0.4[rarr]2.1 [micro]g/dL). Common adverse events (AE) were fatigue, nausea, arthralgia, vomiting, headache, edema, and hypokalemia, possibly due to mineralocorticoid receptor activation by high cortisol. Adrenal insufficiency was reported as an AE in only 2 subjects, but MIFE was decreased or interrupted and glucocorticoids were administered in several cases.

Thirty-four subjects completed 24 weeks; 7 dropped out due to AEs. Mean endometrial thickness increased from 5.9 to 15.7 mm in pre- and from 2.8 to 7.4 mm in postmenopausal women; 2 premenopausal women had prolonged menometrorrhagia. Reversible decreases in HDL-cholesterol and increases in TSH were observed in several subjects.

CONCLUSION: Treatment of subjects with refractory Cushing's syndrome with the GR antagonist mifepristone over 24 weeks produced significant clinical and metabolic improvement with an acceptable risk-benefit profile.

Sources of Research Support: Corcept Therapeutics.

Disclosures: MF: Principal Investigator, Corcept Therapeutics; Novartis Pharmaceuticals; Consultant, Novartis Pharmaceuticals. BMKB: Principal Investigator, Corcept Therapeutics; Clinical Researcher, Novartis Pharmaceuticals; Consultant, Novartis Pharmaceuticals. JWF: Principal Investigator, Corcept Therapeutics; Consultant, Corcept Therapeutics; Novartis Pharmaceuticals. MEM: Principal Investigator, Corcept Therapeutics; Clinical Researcher, Ipsen; Tercica; Advisory Group Member, Novartis Pharmaceuticals. DES: Principal Investigator, Corcept Therapeutics. CG: Employee, Corcept Therapeutics.

Pub # OR09-6
Session Information ORAL SESSION: CLINICAL - Will Medical Management Replace Surgery for Cushing Syndrome? (11:15 AM-12:45 PM)
Title Pasireotide (SOM230) Demonstrates Efficacy in Patients with Cushing Disease: Results from a Large, Randomized-Dose, Double-Blind, Phase III Study
Author String A Colao, S Petersenn, J Newell-Price, JW Findling, F Gu, M Maldonado, U Schoenherr, D Mills, LR Salgado, BMK Biller
University of Naples Federico II, Naples, Italy; ENDOC Center for Endocrine Tumors, Hamburg, Germany; The Medical School, University of Sheffield, Sheffield, UK; Medical College of Wisconsin, Milwaukee, WI; Peking Union Medical College Hospital, Beijing, China; Oncology Business Unit, Novartis Pharma AG, Basel, Switzerland; Hospital das Clínicas, University of São Paulo School, São Paulo, Brazil; Massachusetts General Hospital, Boston, MA

Body **Introduction:** Pasireotide is a multireceptor-targeted somatostatin analogue with high affinity for sst5, which is commonly expressed in corticotroph adenomas, thus having potential as therapy for Cushing's disease.
Methods: 162 patients with persistent/recurrent or *de novo* (if not surgical candidates) Cushing's disease were randomized (double-blind) to pasireotide 600[μg] (n=82) or 900[μg] (n=80) sc bid. After 3mo, patients with UFC>2[acute]ULN (ULN: 145nmol/24h) or UFC>baseline were unblinded and the dose increased by 300[μg] bid. All others continued on the same double-blind dose to 6mo. Months 6-12 were open-label with dose titration performed when needed. Primary endpoint: UFC[le]ULN at 6mo without dose up-titration from the randomized dose.
Results: The median percent decrease in UFC from baseline to month 2 was ~50% in both treatment arms and remained stable throughout the study. At 6 months, 14.6% (600[μg]) and 26.3% (900[μg]) of patients met the primary endpoint; at 12 months, 13.4% (600[μg]) and 25.0% (900[μg]) of patients had UFC[le]ULN. Patients with baseline UFC[le]5[acute]ULN were more likely to achieve normalized UFC. Most uncontrolled patients could be identified within 2 months, based on UFC levels. Serum and salivary cortisol and plasma ACTH were also reduced. As mean UFC decreased, clinical signs and symptoms, and QoL improved. The safety profile of pasireotide was similar to that of other somatostatin analogues (mostly transient GI discomfort), except for hyperglycemia; 70% of patients had a hyperglycemia-related AE. Elevated fasting blood glucose and HbA_{1c} were seen soon after pasireotide initiation. Patients without diabetes at baseline had a lower degree of hyperglycemia. Thirteen (8.0%) patients had an AE of hypocortisolism, responsive to dose reduction.
Conclusion: Results from this Phase III study show that pasireotide significantly reduces elevated cortisol levels and provides clinical benefit in patients with Cushing's disease, supporting its potential for use as the first specific pituitary-targeted treatment in this disorder.

Sources of Research Support: Novartis Pharma AG.

Disclosures: SP: Advisory Group Member, Novartis Pharmaceuticals; Ipsen; Speaker, Novartis Pharmaceuticals; Ipsen. JN-P: Consultant, Novartis Pharmaceuticals; Ipsen; Otsuka; Pfizer, Inc. JWF: Investigator, Novartis Pharmaceuticals; Corcept Therapeutics. MM: Employee, Novartis Pharmaceuticals. US: Employee, Novartis Pharmaceuticals. DM: Employee, Novartis Pharmaceuticals. BMKB: Principal Investigator, Corcept Therapeutics; Novartis Pharmaceuticals; Consultant, Novartis Pharmaceuticals. Nothing to Disclose: AC, FG, LRS

Pub # Y1
Session Information ENDOCRINE YEAR IN BASIC/CLINICAL SCIENCE SESSION: CLINICAL - The Year in Bone (11:15 AM - 12:00 PM)
Title The Year in Bone
Author String JP Bilezikian
Columbia University College of Physicians & Surgeons, New York, NY
Body Session supported by:: Lilly USA, LLC

Disclosures: JPB: Advisory Group Member, Merck & Co., Amgen, Eli Lilly & Company, Johnson & Johnson, Novartis Pharmaceuticals; Researcher, NPS.

Pub # W1-1
Session Information WORKSHOP: CLINICAL - What Can Be Unified & What Needs To Be Individualized in Obesity & Type 2 Diabetes? (11:15 AM - 12:00 PM)
Title Pathogenesis
Author String MW Schwartz
University of Washington, Seattle, WA
Body Disclosures: MWS: Advisory Group Member, Merck & Co., Pfizer, Inc., Orexigen.

Pub #	W1-2
Session Information	WORKSHOP: CLINICAL - What Can Be Unified & What Needs To Be Individualized in Obesity & Type 2 Diabetes? (11:15 AM - 12:00 PM)
Title	Treatment
Author String	AB Goldfine Joslin Diabetes Center, Boston, MA
Body	Disclosure Incomplete: ABG

Pub # CDW1

Session Information CAREER DEVELOPMENT WORKSHOP: Career Development Workshop: Setting up a Lab (11:15 AM - 11:45 AM)

Title Setting up a Lab

Author String AV Lee
University of Pittsburgh, Pittsburgh, PA

Body The completion of graduate/postdoctoral training and successful negotiation of an independent research position is often followed by the daunting task of [ldquo]setting up a lab[rdquo]. While many new faculty are outstanding scientists, having published in the highest peer-reviewed journals, most are poorly trained for the multidisciplinary abilities needed to develop a laboratory and subsequent research career. This is obviously a significant conundrum, as the faculty who are trained to be outstanding bench scientists are ultimately not those that actually do the research. As Thomas R. Cech, Ph.D., President of the Howard Hughes Medical Institute said, [ldquo]Their [faculty] success ultimately lies in their ability to guide, lead, and empower others to do their best work.[rdquo]

The last several years have seen a fundamental shift in the realization that development of successful scientist requires teaching not only basic and clinical science, but also other skills such as team building, public speaking, time management, and conflict resolution. Several universities have developed programs to teach these skills (1).

In this discussion I will highlight the skills, sometimes termed [ldquo]scientific management skills[rdquo], that are needed for the successful development of a laboratory and research program. The discussion will focus on:

Developing a leadership style

- Creating a vision
- Instilling values
- Recruiting personnel
- Time management
- Organizational management including lab notebooks
- Mentoring
- Funding and managing a budget

(1)Aschwanden C, Cell 2006; 125:407

Nothing to Disclose: AVL

Pub # CDW2

Session Information CAREER DEVELOPMENT WORKSHOP: Career Development Workshop: Setting up a Clinical Practice (12:00 PM - 12:30 PM)

Title Setting up a Clinical Practice

Author String EG Levy
University of Miami Miller School of Medicine, Miami Beach, FL

Body In this session, I will provide some suggestions and guidelines for setting up and running a successful clinical practice, no matter where your office will be: in your own practice, working for a large clinic, working in an academic faculty practice, or for an HMO. The key to success is to understand what patients are looking for in their doctor and to be able to provide those things in a cost effective manner. Remember, patients do have a choice of doctors, nowadays, and will seek the one who meets their needs.

Nothing to Disclose: EGL

Pub #	ESL1
Session Information	ENDOCRINOLOGY & SOCIETY PLENARY ADDRESS: Endocrinology & Society Session: Biomedical Research & Healthcare Reform (12:45 PM - 1:30 PM)
Title	Endocrinology & Society: Biomedical Research & Healthcare Reform
Author String	D Shalala President, University of Miami and Former Secretary, Health and Human Services
Body	

Pub # P1-1

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Hormonal Regulation of Hypertrophy and Fibrosis Associated with Cardiac and Renal Disease

Author String CS Samuel, C Zhao, TD Hewitson
University of Melbourne, Parkville, Australia; University of Melbourne, Parkville, Australia; Royal Melbourne Hospital, Parkville, Australia

Body Background: Gender can influence the incidence and progression of cardiac and renal disease. The hormones testosterone, estrogen and relaxin are likely contributing factors. Male but not female relaxin gene-knockout (Rln^{-/-}) mice show an age-related left ventricular (LV) and kidney fibrosis, atrial and renal hypertrophy, and related dysfunction from 9 months; providing a suitable animal model to study the role of these hormones in gender-related pathologies. We recently demonstrated in ovariectomized female Rln^{-/-} mice that estrogen did not influence cardiac and renal collagen deposition, but protected against cardiac hypertrophy in synergy with relaxin. We thus hypothesized that testosterone may explain the difference in fibrotic phenotype. Methods: Male Rln^{-/-} and age-matched wild-type (Rln^{+/+}) mice were castrated at 1-month of age and maintained on soy-free food until 12-months old (when cardiac/renal fibrosis is established), before being killed for tissue collection and measurement of organ hypertrophy, collagen concentration and distribution. Subgroups of castrated animals were treated with testosterone or estrogen replacement therapy from 9-12 months of age, while 12-month sham-operated animals (with testes intact) were used as controls. Results: Castration reduced body, heart, LV and kidney weights in both Rln^{-/-} and Rln^{+/+} male mice, and the cardiac/renal fibrosis that was seen in sham Rln^{-/-} animals (all p<0.05 vs respective sham). Testosterone, but not estrogen, normalized heart, LV and kidney weights and organ weight/body weight ratio of castrated animals, and increased cardiac/renal collagen concentration and staining to levels measured in or beyond that of sham Rln^{-/-} mice (all p<0.05 vs respective castrated mice). Furthermore, cardiac expression of TGF- β 1, phosphorylation of its signal transduction molecule Smad2 and expression of α -smooth muscle actin (a marker of myofibroblast differentiation) paralleled the above changes (all p<0.05 vs respective castrated mice). Conversely, estrogen restored atrial hypertrophy, glomerular density and volume. Conclusions: These findings suggest that the gender-specific fibrosis in male Rln^{-/-} mice is primarily due to the detrimental affects of testosterone, while estrogen, in synergy with relaxin, regulates age-related cardiac/renal growth. Further evaluation of the opposing roles of testosterone and relaxin may contribute to our understanding of the differences that occur between men and women.

Sources of Research Support: NHFA/NHMRC RD Wright Fellowship CR06M2749/454636 awarded to CSS.

Nothing to Disclose: CSS, CZ, TDH

Pub # P1-2
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)
Title Physiological Effects of Androgens on Human Vascular Endothelial and Smooth Muscle Cells in Culture
Author String L Nheu, S Ling, L Nazareth, RZ Luo, PA Komesaroff
Monash, Melbourne, Australia

Body The fact that men are more susceptible to atherosclerosis than women suggests a role for sex hormones in its pathological formation. The effects of androgens on the physiology of vascular cells and on underlying molecular pathways have been much less studied than those of estrogens. This study examined effects of the androgens testosterone (T), dihydrotestosterone (DHT) and dehydroepiandrosterone (DHEA) on human vascular endothelial cells (EC) and smooth muscle cells (VSMC) cultured in vitro. The cells were incubated with hormones at concentrations ranging from physiological (5nM) to supraphysiological (50nM). DNA synthesis was assessed by assay of [3H]-thymidine incorporation, cell growth and death by cell number counting, collagen synthesis by assay of [3H]-proline incorporation, the expression of the constituents of an extensive panel of gene proteins by Western blotting analysis and dependence on classical pathways by the effects of the AR antagonist flutamide (100nM). It was shown that: (1) T stimulates DNA synthesis and cell growth in EC in a concentration-dependent manner ($p=0.005$ & 0.03 at 5nM & 50nM respectively) via an AR independent mechanism; DHT inhibits DNA synthesis and cell growth ($p=0.04$ & 0.03) in an AR dependent manner; but DHEA does not significantly affect EC DNA synthesis and growth; (2) T and DHT, but not DHEA, stimulate VSMC proliferation ($p=0.003$) in a concentration-dependent manner, the effect being independent of AR for T and AR dependent for DHT; (3) no hormone significantly changes collagen synthesis in VSMC despite the increased cell numbers associated with T and DHT; and (4) T activates MAP kinase ERK1/2 activity via AR independent mechanism(s) but expression of proteins Egr-1, Sp-1, PKC α , PKC β 1, ICAM-1, VCAM-1, JNK, p38, Akt, caspase-1, Bcl-2 or NF-[kappa]B is unaffected by any hormonal treatment. We conclude that androgens produce multiple and sometimes conflicting effects on vascular cells via both AR dependent and independent mechanisms. Some effects, such as enhancement of EC growth by T and protection of EC injury by DHEA (previously shown), are potentially beneficial in that they may inhibit aspects of the atherosclerotic process, while others, such as T stimulation of VSMC proliferation, are potentially harmful in that they may promote it. Further study is needed to elucidate the specific mechanisms by which these in vitro effects are controlled and to determine whether they can be independently modified.

Sources of Research Support: Research Project Grant (No. 1610001) of National Institute of Complementary Medicine of Australia.

Nothing to Disclose: LN, SL, LN, RZL, PAK

Pub # P1-3

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Androgen-Estrogen Balance, SHBG, and Cognitive Trajectories in Older Men and Women

Author String JS Lee, D Harvey, O Carmichael, D Mungas, B Reed, J Olichney, B Kaur, J Miller, C DeCarli
Kaiser Permanente, Oakland, CA; University of California Davis, Sacramento, CA; University of California, Sacramento, CA; University of California Davis, Sacramento, CA; University of California Davis, Sacramento, CA

Body

Objective: To assess associations between androgen-estrogen balance and sex hormone binding globulin (SHBG) and cognitive decline.

Background: Much research focuses on the cognitive roles of estrogen in women and androgen in men. Metabolism of endogenous estrogen, androgen, and their major binding globulin, SHBG, are inter-related. Yet, the roles of endogenous androgen-estrogen balance and SHBG on cognition in older women and men remain unclear.

Design/Methods: We measured estradiol (E2), estrone, testosterone (T), androstenedione, and SHBG, in banked serum collected at baseline in an existing cohort of 215 older adults (80 males and 135 females; mean age = 75.3 years). Eligible participants were evaluated using standardized cognitive assessment protocols, within 6 months of blood collection. They were followed longitudinally, with at least two evaluations during a median follow-up of 3.2 years, for episodic memory and executive function. Gender-specific repeated-measures random effects models were used, with and without adjustment for demographics, ApoE4 genotype, and a composite vascular risk score. Those taking sex hormone altering therapies were excluded.

Results: Of the 215 participants, 38 (17.7%) had dementia, 65 (30.2%) had mild cognitive impairment, and 112 (52.1%) had normal cognition. Women with high SHBG levels had worse episodic memory at baseline and faster decline of executive function, in all models. Baseline episodic memory score was 0.2 points lower and executive function had a 0.05-point faster decline, for every 1 standard deviation (S.D.) increase in baseline SHBG (P<0.01). In contrast, men with higher E2/T ratio at baseline had worse episodic memory, with a 0.2 lower score per 1 S.D. increase in the ratio. With additional adjustment for BMI, associations were similar. No other associations were observed consistently across all models.

Conclusion: The roles of endogenous sex hormones and SHBG on cognitive performance vary by gender and the particular cognitive domain. Women with higher circulating levels of SHBG have a worse episodic memory and faster decline in executive function. Men with higher endogenous estrogen, relative to androgen, levels have worse episodic memory.

Sources of Research Support: NIH/NIA P30 AG010129, NIH/NCRR UL1 RR024146 (Lee), NIH K01 AG 030514 (Carmichael).

Nothing to Disclose: JSL, DH, OC, DM, BR, JO, BK, JM, CD

Pub # P1-4

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title The Effect of Testosterone on the Regeneration of Young and Old Skeletal Muscle

Author String C Serra, F Tangherlini, S Rudy, D Lee, A Zhang, G Toraldo, R Jasuja, S Bhasin
Boston Medical Center, Boston, MA

Body Background: Aging is associated with a loss of muscle mass and strength, reduced satellite cell number, and lower regenerative potential after skeletal muscle injury. Testosterone increases muscle mass and strength, and satellite cell number in humans. However, the effects of testosterone on the regenerative potential of skeletal muscle, especially in older Mammals, are unknown. We investigated the effect of testosterone on skeletal muscle regeneration of young (2 month-old) and old (24 month-old) male mice.
Methods: We randomly assigned mice to three experimental groups (n=5/group): sham-operated, vehicle-treated (Sham); castrated, vehicle-treated (Cast); castrated supplemented with testosterone propionate (Tp) (Cast Tp). After 12 days of treatment, muscle injury was induced by injecting 20 microL of 10 microM cardiotoxin in the *tibialis anterior* muscle. Mice were euthanized 2, 3, 4 and 9 days after muscle injury.
Results: Testosterone rescued body mass in both young and old castrated mice. Testosterone supplementation was associated with an increased number of proliferating satellite cells in regenerating muscle of young Cast mice 2 days post-injury, compared to Cast control. Testosterone supplementation also increased the number and cross-sectional area (CSA) of embryonic-myosin heavy chain⁺ (emb-MyHC⁺) regenerating fibers in both young and old Cast mice 4 days post-injury. The increase in emb-MyHC⁺ fibers in young mice matched the lower number of proliferating satellite cells 4 days post-injury, indicating an androgen-dependent increase in myoblast fusion and differentiation. Testosterone increased the muscle fiber CSA of the un-injured *tibialis anterior* muscle of both young and old Cast mice. Compared to Cast controls, the *levator ani* muscle of testosterone-treated mice had higher mass, satellite cell number, myonuclear number, and fiber CSA in young as well as old mice. When single muscle fibers isolated from the *levator ani* muscle of young mice were cultured *in vitro* for three days, testosterone supplementation was associated with a marked increase in the number of delaminating satellite cells *in vitro*, compared to fibers isolated from Cast mice.
Conclusions: Testosterone affects satellite cell activation and proliferation, and improves muscle regeneration. These data suggest potential application of androgens to improve the regenerating potential of old skeletal muscle after injury.

Nothing to Disclose: CS, FT, SR, DL, AZ, GT, RJ, SB

Pub # P1-5

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Testosterone Amelioration of High-Fat Diet-Induced Non-Alcoholic Fatty Liver Disease (NAFLD) Is Not Accompanied by Changes in Insulin-Dependent Lipogenic and Lipolytic Enzymes

Author String Y Jia, L Nikolaenko, CCL Wang, M Diaz-Arjonilla, Y Lue, C Chong, S Laurel, SW French, JK Yee, PWN Lee, RS Swerdloff
LA BioMed&Harbor-UCLA Medical Center, Torrance, CA; LA BioMed&Harbor-UCLA Medical Center, Torrance, CA; LA BioMed&Harbor-UCLA Medical Center, Torrance, CA

Body Objective: NAFLD is the leading cause of hepatic dysfunction and is associated with obesity, diabetes, insulin resistance and Metabolic Syndrome. Testosterone (T) deficiency is a risk factor for developing the metabolic syndrome type 2 diabetes, but the role of T deficiency in hepatic steatosis has not been well studied. We established a rat model of hepatic steatosis to investigate the effects of testosterone (T) on pathogenesis of NAFLD. We showed that liver histopathology revealed a severe micro- and macro-vesicular accumulation of fat in hepatocytes with multiple inflammatory foci of castrated rats fed HFD that was attenuated by T replacement in castrated rat (Nikolaenko et al. ENDO 2010). We studied three critical insulin-regulated proteins in liver and omental adipose tissue in this rat model, namely fatty acid synthase (FAS), adipose triglyceride lipase (ATGL), and hormone sensitive lipase (HSL) to assess whether they contributed to the development of hepatic steatosis.
Methods: Male rats were randomly placed into four groups (n=8 per group): castrated rats on high-fat diet (HFD), castrated rats with T replacement on HFD, intact rats on HFD, and intact rats on regular chow diet (RCD). The HFD provided 71% energy from fat; RCD provided 16% of energy from fat. The rats were fed ad libitum for 15 weeks then animals had been sacrificed and liver tissue been collected and kept at -80C. Western blot was used to determine the changes of FAS, ATGL, and HSL in liver and omental adipose tissue.
Results: Based on western blot analyses there was no increase FAS expression in the liver or the adipose tissue in any group ATGL or HSL protein expression was not different in any of the groups.
Conclusion: T replacement of castrated rats with HFD reduces hepatic steatosis without significantly changing lipogenic or lipolytic enzyme expression in the liver tissue and omental adipose tissue. As all these enzymes are regulated by insulin, insulin resistance may not play an important role in the protective effect of T in this hepatic steatosis rat model.

Disclosures: RSS: Principal Investigator, Clarus; Abbott Laboratories. Nothing to Disclose: YJ, LN, CCLW, MD-A, YL, CC, SL, SWF, JKY, PWNL

Pub # P1-6

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Gonadectomy Attenuates Starvation-Induced Autophagy in Female but Not Male Rat Liver

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Body Autophagy is a cellular degradation pathway for removal of misfolded proteins and organelles that are damaged by age or stress, thereby maintaining cellular homeostasis and providing nutrients during times of starvation. Although low levels of autophagy are necessary for normal cell function, pathological changes in autophagy are associated with a number of diseases including cardiovascular disease, cancers, neurodegeneration and diabetes. Most autophagy research has been conducted in male animals or in cultured cells; thus it is unclear if sex differences in autophagy exist and if gonadal hormones regulate autophagy. There are sex differences in many disease processes characterized by dysregulation of autophagy, and hypogonadism is associated with an increased risk of diseases that involve dysregulation of autophagy. Therefore, we hypothesized that sex and gonadal status affect liver autophagy. To test this hypothesis, we examined cellular markers of autophagy, beclin-1 and LC3, in the liver of four groups of young adult (2 mo) male and female rats: gonadally intact, fed and fasted; gonadectomized (GDX), fed and fasted. Fasted rats were food-deprived overnight. Intact females were fasted on the night of proestrus and killed on the morning of estrus. Whole cell liver lysates were tested for beclin-1, LC3I and LC3II protein abundance via western blot. β -tubulin was used as a loading control. As expected, fasting significantly increased hepatic beclin-1 and LC3I/LC3II abundance in intact male and female rats ($P < 0.05$). Fasted male GDX rats also exhibited elevated beclin-1 and LC3I/LC3II expression in the liver, but this was not true for females ($P < 0.01$). In addition, GDX males had significantly higher beclin-1 protein levels than females and intact males ($P < 0.01$). Liver from GDX males also had higher LC3I/LC3II abundance than tissue from GDX females ($P < 0.05$). These data suggest an interesting sex difference and a role for gonadal hormones in autophagy. Although both intact male and female and GDX male rats display the expected increase in autophagy following food deprivation, GDX female rats do not show an increase in autophagy following food deprivation. Additionally, GDX male rat livers have higher basal levels of autophagy than females. These data suggest that autophagy in the liver is affected by sex and gonadal steroids.

Sources of Research Support: Albert Einstein College of Medicine and The Einstein Aging Research (T32) Training Grant.

Nothing to Disclose: JS, SDB, SJ, AME, GN-P

Pub # P1-7
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title The GPER1 Agonist G-1 Attenuates Aortic Sprouting in Mice

Author String A Holm, P-O Grande, B Olde, F Leeb-Lundberg, B-O Nilsson
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Body Estrogen exerts its actions via the two nuclear estrogen receptors named ER α and ER β , but non-nuclear estrogenic signalling has also been suggested. The G protein-coupled estrogen receptor GPER-1, formerly known as GPR30, has been implicated in estrogen signalling, but the physiological functions of GPER1 are not fully understood. A synthetic agonist for GPER1, named G-1, has been developed (1). *In vivo* experiment in rats showed that acute administration of G-1 slightly reduced blood pressure (2). Furthermore, chronic (2 weeks) administration of G-1 via an osmotic minipump lowered blood pressure in ovariectomized hypertensive mRen2. Lewis rats (3). These reports suggest that G-1 reduces blood pressure. G-1 has also been shown to attenuate proliferation of cultured vascular smooth muscle cells (2) and urinary bladder epithelial cells (4). In order to further elucidate the cardiovascular role of GPER1, we investigated the effects of G-1 on blood pressure, heart rate and angiogenesis in mice.

Intravenous injection of a bolus dose of G-1 (0.5, 5 and 50 [μ g/kg) had no effect on blood pressure as determined at 3 min after the injection in intact male or female mice. Heart rate was also not perturbed by G-1. We then evaluated the cardiovascular effects of G-1 in ovariectomized mice after removal of endogenous estrogen production. G-1 also had no effect on blood pressure or heart rate in these mice. As positive control, intravenous injection of noradrenaline (10 [μ g/kg) was shown to increase blood pressure by about 50%. Aortic rings from mice were placed in a cell/tissue culture incubator for 10 days with or without G-1 in Matrigel. G-1 at a dose (3 [μ M]), which is comparable to that used in *in vivo* experiments, completely attenuated aortic sprouting. The sprouting resumed upon G-1 removal and no effect was observed on cell viability as determined by Trypan-blue staining. The anti-angiogenic G-1 effect was probably not due to altered VEGF production or VEGFR expression because VEGF-A, VEGFR1 and VEGFR2 mRNA levels were unaffected by G-1. In summary these data suggest that the GPER-1 agonist G-1 reduces angiogenesis via a VEGF-independent mechanism but has no effect on blood pressure and heart rate.

(1) Bologna CG et al., Nature chemical biology 2006; 2:207

(2) Haas E et al., Circulation research 2009; 104:288

(3) Lindsey SH et al., Endocrinology 2009; 150:3753

(4) Teng J et al., Endocrinology 2008; 149:4024

Nothing to Disclose: AH, P-OG, BO, FL-L, B-ON

Pub # P1-8
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title 17 β -Estradiol Increases Amiloride-Sensitive Currents and PKC[delta] Activity in M1 Cells

Author String YR Yusef, BJ Harvey, W Thomas
RCSI, Dublin, Ireland

Body 17 β -estradiol (E2), the most abundant estrogen naturally occurring in the female body, regulates chloride secretion in distal portions of the colon in a gender-specific manner. E2 exerts its effect via inhibition of basolateral K⁺ channels (1). The anti-secretory effect of E2 could provide an explanation for the fluid retention observed in females during periods of high circulating plasma estrogen (1). The effect of E2 in other organs contributing to the fluid homeostasis has not been characterised. In the kidney, specifically in distal parts of the nephron such as the cortical collecting ducts, the modulation of Na⁺ re-absorption by E2 could contribute to fluid conservation during periods of high circulating plasma estrogen.

The aim of this study was to determine whether E2 has an effect on Na⁺ re-absorption in the M1 cortical collecting duct cell line, an extensively used model for the principal cells of the cortical collecting ducts and also to characterize the molecular mechanisms underlying the effect of E2. We found that treatment with E2 (25nM) for 15 min increased the amplitude of the amiloride sensitive current in M1 cells (controls 1.47 \pm 0.37, E2-treated 2.80 \pm 0.42) grown as polarized monolayers, an effect not attributable to changes in Na⁺/K⁺ATPase activity (ouabain sensitive currents). Previous work from our laboratory has shown that the anti-secretory effect of E2 in distal colon is PKC[delta]-dependent (1). To test the hypothesis that E2-induced Na⁺ re-absorption in M1 cells is also PKC[delta]-dependent, we investigated the phosphorylation state of the PKC [delta] autophosphorylation residue Ser643 following E2 treatment. E2 stimulated PKC[delta] autophosphorylation in a dose-dependent manner and within a similar time frame as the effect on the amiloride-sensitive current. We found that PKC[delta] autophosphorylation was also stimulated by the ER α agonist PPT (1nM) but not by the ER β agonist DPN (5nM). The stimulatory effect of E2 on PKC[delta] autophosphorylation in M1 cells is blocked by pre-treatment with the matrix metalloproteinase (MMP) inhibitor GM6001 (1[μ]M) and with the EGFR inhibitor AG1478 (10[μ]M).

In conclusion, E2 treatment rapidly and concurrently increased the amplitude of the amiloride-sensitive current and stimulated PKC[delta] activity in M1 cells. This physiological response was initiated by the interaction of E2 with ER α and required the MMP-mediated transactivation of EGFR.

(1) O'Mahony et al., J Biol Chem 2007; 282(34):24563-73

Sources of Research Support: Higher Education Authority of Ireland (PRTL14) through the National Biophotonics and Imaging Platform.

Nothing to Disclose: YRY, BJH, WT

Pub # P1-9

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Hormonal Effects on Inflammation in the Pubertal vs. Adult Mammary Gland

Author String RC Schwartz, Y Zhao, YS Tan, W Wang, LA Zustiak, SZ Haslam
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Body We recently demonstrated that progesterone (P) induces an inflammatory response in the mammary epithelium. The role of hormones, P in particular, in mediating an inflammatory response in the mammary gland was not previously recognized. We found that progestin treatment of cultured mammary epithelial cells induces several proinflammatory gene products implicated in the induction of inflammation. These products increase expression of proinflammatory cytokines in monocytes and neutrophils, and promote monocyte and neutrophil recruitment and adhesion. Inflammatory processes are required for tissue remodeling and angiogenesis, which are essential for normal mammary gland development. However, in the context of mammary carcinogenesis, these processes can also promote tumor development.

Further in vivo analysis of the inflammatory effects of P, estrogen (E), and E+P in the mammary glands of pubertal and virgin adult BALB/c mice show that these hormone treatments induce an even broader range of proinflammatory products in intact mammary glands than observed in cell culture. Different, but overlapping, sets of cytokine and chemokine RNAs were observed with the various hormone treatments and at the pubertal and adult stages of development. Common to P and E+P treatments of pubertal and adult mice is induction of RANKL, IL-17B, and TNF α . RANKL induction is particularly robust, and can induce SAA, a proinflammatory product, in cultured mammary epithelial cells and in a mammary epithelial cell line. Treatments that include E specifically induce IL-10, an anti-inflammatory cytokine.

One common property of RANKL, IL-17B, and TNF α is the ability to induce activation of NF-[kappa]B, a critical factor in transcriptional induction of many proinflammatory products. The broader range of cytokines and chemokines in the whole gland vs. cultured epithelial cells suggests participation of both epithelial and stromal elements in their expression. Our findings suggest a chain of events where P induces RANKL, an NF-[kappa]B activator, which in turn induces various other proinflammatory cytokines and chemokines that recruit macrophages and eosinophils to the peri-epithelial stroma. The induction of IL-10 with treatments including E may moderate these effects. We confirmed that both macrophages and eosinophils are recruited on hormonal treatment, with both E and P being potent in this regard at puberty. At adulthood, only P potently recruits leukocytes, and E actually moderates the effects of P.

Sources of Research Support: Breast Cancer and the Environment Research Centers Grant U01 ES/CA 012800 from the National Institute of Environment Health Science (NIEHS) and the National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health and Human Services. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS or NCI, NIH

Nothing to Disclose: RCS, YZ, YST, WW, LAZ, SZH

Pub # P1-10

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title 17 β -Estradiol Increases Expression of Apurinic Endonuclease (Ape1): A Mechanism To Confer Neuroprotection?

Author String AK Dietrich, AM Nardulli
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Body Stroke is the third leading cause of death and a major source of permanent disability in the United States. The fact that postmenopausal women are at greater risk of suffering a stroke than premenopausal women suggests that circulating ovarian hormones may be required to maintain optimal brain cell function and reduce the risk of stroke. In support of this idea, a number of studies have shown that 17 β -estradiol (E2) attenuates ischemia-induced damage in rodent models of stroke. Interestingly, our laboratory recently showed that E2 increases expression of oxidative stress response proteins in cultured mammary cells and other laboratories have shown that overexpression of oxidative stress response proteins decreases ischemia-induced brain injury. Thus, we hypothesized that E2 may in part mediate its protective effects in the brain by increasing oxidative stress response protein expression.

Our studies focused on the oxidative stress response protein apurinic endonuclease (Ape1), which is also known as redox factor-1. Ape1 is a multifunctional protein that, in addition to reducing a number of transcription factors, is also required for DNA repair. Importantly, overexpression of Ape1 in rodents reduces DNA fragmentation and infarct volume in the cerebral cortex after an ischemic event.

To determine whether E2 altered Ape1 expression in the cerebral cortex, we utilized mouse brain slice cultures, which maintain many of the architectural features and cellular networks found in the intact brain, but permit a more direct assessment of the effects of E2 on the brain than is possible in intact animals. Immunofluorescent studies demonstrated that estrogen receptor α and Ape1 were expressed in the nuclei of cortical neurons and that E2 treatment of brain slice cultures increased Ape1 expression in the cerebral cortex. Additionally, quantitative Western blot analysis using extracts from brain slice cultures confirmed that E2 increased Ape1 expression. Our studies suggest that the E2-induced expression of Ape1 helps to protect the cerebral cortex from an ischemic insult and defines a novel mechanism by which E2 may mediate its neuroprotective effects.

Sources of Research Support: NIH grant R01 DK 53884.

Nothing to Disclose: AKD, AMN

Pub # P1-11

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Involvement of NF[κ]B Signaling Pathway in the Proapoptotic Action of Estrogens in the Anterior Pituitary Gland

Author String G Eijo, S Zarate, G Jaita, J Ferraris, ML Magri, D Radl, V Zaldivar, V Boti, D Pisera, A Seilicovich
School of Medicine, Buenos Aires University, Buenos Aires, Argentina

Body Nuclear Factor kappa B (NF[κ]B), a transcription factor activated by several stimuli including TNF- α and lipopolysaccharide (LPS), induces the expression of genes involved in cell survival. Activation of estrogen receptors mediates the inhibition of the NF[κ]B pathway in various cell types. Since in anterior pituitary (AP) cells, estrogens exert a sensitizing effect to proapoptotic stimuli, we investigated whether the estrogenic action involves inhibition of this pathway. We determined the effect of 17 β -estradiol (E₂) on NF[κ]B/p65 and p50 nuclear translocation in primary cultures of AP cells from ovariectomized (OVX) rats. As determined by Western blot, E₂ decreased TNF- α -induced NF[κ]B/p65 and p50 translocation in cultured AP cells. E₂ administration (200 mg/kg for 2 days) to OVX rats reduced LPS-induced activation of NF[κ]B/p65 and p50 in the anterior pituitary gland as well as Bcl-xL expression (C: 1.0 \pm 0.0, LPS: 1.4 \pm 0.1, E₂: 1.2 \pm 0.1, E₂+LPS: 1.2 \pm 0.1; p < 0.01; ANOVA). To investigate whether inhibition of the NF[κ]B pathway sensitizes AP cells to proapoptotic stimuli, the cells were incubated with BAY 11-7082 (Bay), an inhibitor of NF[κ]B pathway. In the presence of Bay at a concentration that does not induce apoptosis (1 [micro]M), TNF- α increased the percentage of TUNEL positive-lactotropes (C: 3.8%, TNF- α : 4.1%, Bay: 3.6%, Bay + TNF- α : 17.5%; p < 0.01; [chi]²) and somatotropes (C: 3.8%, TNF- α : 3.6%, Bay: 3.4%, Bay + TNF- α : 7.5%; p < 0.01; [chi]²) from OVX rats. E₂ enhanced the sensitizing action of Bay in TNF- α -induced apoptosis of lactotropes (TNF- α : 4.1%, Bay + TNF- α : 17.2%, E₂ + TNF- α : 15.7%, Bay + E₂ + TNF- α : 37.8%; p < 0.01; [chi]²) but not of somatotropes. LPS administration also increased the percentage of apoptosis in AP cells when OVX rats were treated with Bay (5 mg/Kg) (V: 9.2 \pm 0.9, LPS: 10.4 \pm 0.6, Bay: 8.9 \pm 0.5, Bay + LPS: 19.9 \pm 0.3; p < 0.01; ANOVA). These results suggest that E₂ sensitizes AP cells to proapoptotic stimuli by decreasing the activity of the NF[κ]B pathway. Additive effects of E₂ and Bay suggest that additional mechanisms contribute to TNF- α -induced apoptosis in lactotropes.

Sources of Research Support: Grants from Agencia Nacional de Promoción, Científica y Tecnológica; National Research Council, Argentina; Buenos Aires University.

Nothing to Disclose: GE, SZ, GJ, JF, MLM, DR, VZ, VB, DP, AS

Pub # P1-12

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Estrogen Action on Iron Metabolism

Author String Y Ikeda, S Tajima, N Yamano, Y Kihira, K Ishizawa, S Tomita, K Tsuchiya, T Tamaki
The University of Tokushima Graduate School of Health Biosciences, Tokushima, Japan; The University of Tokushima Graduate School of Health Biosciences, Tokushima, Japan

Body
Background: Iron is an essential trace metal for living body. On the other hand, excess iron reacts with hydrogen peroxide and products hydroxyl radicals via Fenton reaction, causing oxidative stress. Therefore iron is suggested to be involved in occurrence and progression of various diseases. The Body iron storage is generally lower in female than in male, while the iron store is elevated in women after menopause and there are no difference of iron storage between men and women. It is normally thought that women lost iron during their reproductive years due to monthly menstruation. Recently sex hormone is demonstrated to exert many actions in various organs, however the effect of sex hormone on physiological iron homeostasis is little known. In this study, we investigate the action of female sex hormone, estrogen, on iron metabolism.
Methods and Results: In an in vitro study, 17 β -estradiol (E2) increased the mRNA level of hepcidin, a liver-derived iron regulatory protein, in HepG2 cells in a dose-dependent manner. This E2-induced hepatic hepcidin upregulation was not suppressed by ICI 182,720, an inhibitor of estrogen receptor, meanwhile ICI 182,720 augmented hepcidin expression. ICI 182,720, as well as E2, has an agonist effect of G protein receptor 30 (GPR30), the seven-transmembrane estrogen receptor. Indeed the agonist of GPR30 stimulated hepcidin expression, and E2-induced hepcidin expression was abolished by GPR30 siRNA induction. In an in vivo study, ovariectomized female mice presented hepatic hepcidin down-regulation, and the up-regulated expression of divalent-metal transporter-1 (DMT-1), an iron import protein, and ferroportin, an iron export protein, in duodenum compared to sham-operated female mice.
Conclusion: These findings suggest that female sex hormone directly participates in iron metabolism through hepcidin regulation independently of monthly menstruation.

Nothing to Disclose: YI, ST, NY, YK, KI, ST, KT, TT

Pub # P1-13

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Is Estradiol Required for Cocaine Sensitization in Female Rats?

Author String YM Torres-Diaz, JL Agosto, AC Segarra
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Body Estradiol plays a major role in the control of sexual, emotional and cognitive behaviors in females. It also participates in mediating sex differences in the response to drugs of abuse. Previous studies in our laboratory show that only females with estradiol develop sensitization to 5 daily injections of cocaine (15 mg/kg). This study was designed to investigate if varying the dose, length of treatment or context of cocaine injections induces behavioral sensitization in OVX rats. Rats were injected with a lower [10mg/kg] or higher [30mg/kg] dose of cocaine and the number of daily cocaine injections increased from 5 to 10. Also, to facilitate drug-context associations, OVX and OVX-EB rats were injected daily in the same activity chamber where locomotor activity was recorded. Varying the dose and length of cocaine did not affect sensitization, OVX rat did not become sensitized to cocaine. However, strengthening drug-context associations induced a slight sensitization in OVX rats. These data hint that exposure to same context is associated with drug-induced neural plasticity in female rats and that estradiol may be enhancing memory of drug related cues and in this way enhancing sensitization.

Sources of Research Support: NINDS(SNRP:U54NS39405); NIGMS(RISE:R25GM061838; SCORE:S06GM08224).

Nothing to Disclose: YMT-D, JLA, ACS

Pub # P1-14

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Estrogen Administration Has a Protective Role in Demyelination, Possibly through the Activation of Galanin and Its GalR1 and GalR2 Receptors

Author String L Zhang, S Wu, A Schroedter, J Kong, ME Vrontakis
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Body Multiple sclerosis (MS) is the most common cause of neurological disability affecting young people. Pregnancy appears to have a protective effect on women with MS. This remission is correlated with a decrease in the number and size of active white matter lesions. Galanin(Gal)is a 29/30 amino acid neuropeptide widely distributed in the peripheral and central nervous system. Gal has been shown to have a neuroprotective effect and it is dramatically increased after nerve injury in several systems. There is also strong evidence that Gal is synthesized in myelin producing glial cells. A distinctive feature of Gal gene expression is its up regulation by estrogens. In this study, we investigated the role of estrogen combined with Gal over expression in myelin protective effects in the cuprizone (CPZ) demyelination model of MS by using Gal transgenic (Gal-Tg) mice. Both wild type (WT) and Gal-tg mice were placed into 4 groups: 1. Control group with no treatment of 17 β -estradiol (E2) and CPZ, 2. E2 group with the treatment of E2 only, 3. 6wCPZ group with the 6-week CPZ diet only and 4. E2+6wCPZ group with the combination of administration of E2 and CPZ. Demyelination was measured by immunostaining to myelin basic protein (MBP). Expression of Gal and its three receptors during demyelination was quantitatively determined by comparative Cq Real-time PCR. Our results demonstrated that over-expression of Gal in Gal-Tg mice significantly inhibited CPZ-induced demyelination compared to WT mice. Furthermore, combined with estrogen Gal-Tg mice in E2+6wCPZ showed an even much better protective effect compared to other groups. Moreover, we have observed that the expression levels of Gal were dramatically increased in pituitary and corpus callosum, while to a much lesser extent in the hypothalamus, in the E2 treated groups. In addition, the abundance of GalR1 and GalR2 receptors significantly changed after CPZ insult as well as E2 stimulation. Together, these data suggest a putative role for Gal combined with E2 in preventing myelin damage. The regional differential activation of Gal and its receptors in demyelination processes suggests the potential pathways of E2 in improving demyelination diseases, such as Multiple Sclerosis.

Nothing to Disclose: LZ, SW, AS, JK, MEV

Pub # P1-15
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title A Novel 17 β -Estradiol Prodrug for the Treatment of Menopausal Symptoms in Animal Models of Hot Flush and Depression

Author String I Merckenthaler, M Arad, T Gould, K Tatrai-Prokai, L Prokai
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Body **Background:** The major menopausal symptoms women seek help for include hot flushes and depression/anxiety. Estrogen therapy has been the treatment of choice to alleviate these symptoms; however, many women cannot take estrogens. Therefore, there is a huge unmet need for novel, efficacious, and safe therapies. Para-quinol of 17 β -estradiol (DHED, based on its chemical structure) is selectively converted to 17 β -estradiol (E2) in the mammalian brain, however, such conversion does not occur in the peripheral tissue including the uterus, breast, or the pituitary gland. Thus, DHED's action is restricted to the brain without causing many of the side effects that can be observed following chronic treatment with estrogens. The current studies were designed to test whether DHED can alleviate hot flushes and exhibit antidepressant and anxiolytic properties in animal models.

Methods: To evaluate the effect of DHED on hot flushes, the morphine-dependent rat model was utilized (Merckenthaler et al., 1998). DHED was administered orally at 10.0, 30.0, and 100.0 ug/kg doses to ovariectomized (OVX) rats for 8 days. Following morphine withdrawal, tail skin temperature rise (hot flush) was measured. To evaluate DHED's effect on depression/anxiety, OVX female C57BL/6J mice were tested in several procedures measuring depressive- and anxiety- like behaviors including Forced Swimming Test (FST), Tail Suspension Test (TST), Learned Helplessness (LH), Open Field (OF), and Elevated Plus Maze (EPM). Mice were administered subcutaneously with 0, 1, 3 or 10 [micro]g/kg of DHED or 25 [micro]g/kg of E2 in acute, sub-chronic, and chronic paradigms.

Results: Similarly to E2, DHED reduced naloxone-induced tail skin temperature rise in the rat hot flush model. Importantly, the most efficacious dose of DHED (30 ug/kg) was about six times less than that of ethynyl estradiol. In the mouse models of depression/anxiety, DHED as well as E2 resulted in reduced immobility time in the FST and had no effect on locomotor activity in the OF. However, DHED increased the time animal spent in the center of the field as well as in the open arms of the EPM. DHED also reduced immobility time in the TST and reduced despair-like behavior in the LH. In all procedures, the effects of DHED resembled the effects of E2 but while E2 exhibited uterotrophic activity, DHED did to stimulate the uterus.

Conclusion: The use of a CNS-specific DHED may provide a novel and promising treatment for women who suffer from menopausal symptoms.

Merckenthaler I, Funkhouser JM, Carver JM, Lundeen SG, Ghosh K, Winneker RC. The effect of estrogens and antiestrogens in a rat model of hot flush. *Maturitas* 30:307-316, 1998

Sources of Research Support: NIH grants 1R01AG031535-01A2, 3R01AG031535-01A2S1, and AG031535.

Nothing to Disclose: IM, MA, TG, KT-P, LP

Pub # P1-16
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)
Title An Evaluation of Bazedoxifene Acetate Alone and in Combination with Conjugated Equine Estrogens on the Breast of Surgically Postmenopausal Monkeys
Author String KF Ethun, CE Wood, TC Register, JM Cline, SE Appt, TB Clarkson
Wake Forest University School of Medicine, Winston-Salem, NC

Body Concerns of breast cancer risk with conjugated equine estrogens (CEE) and medroxyprogesterone have generated interest in finding an alternative for the progestin component in postmenopausal estrogen co-therapy. Bazedoxifene acetate (BZA) is a new selective estrogen receptor modulator (SERM) currently under investigation as a possible choice for this role. The primary objective of this study was to determine if BZA would antagonize the proliferative effects of CEE on breast epithelial tissue, while having no breast stimulatory effects when administered alone. Here, we present data from a randomized, placebo-controlled, 24 month, multi-organ system nonhuman primate translational trial. Ninety-five adult cynomolgus monkeys (*Macaca fascicularis*) were ovariectomized and randomized into social groups to receive placebo (control) or treatment with BZA 20 mg, CEE 0.45 mg, BZA 20 mg + CEE 0.45 mg (doses expressed as women's daily equivalents). Following 6 months of treatment, breast biopsies were collected for histomorphometry (breast epithelial area), immunohistochemistry for the proliferation marker Ki67, and gene expression studies including gene microarray analyses. Those animals receiving BZA+CEE and BZA had significantly less breast epithelial area than those animals consuming CEE alone ($p < 0.05$ for both) and nearly equivalent breast epithelial area to those animals receiving no hormone therapy. Ki67 protein expression within the terminal ducts showed a similar pattern in which the number of positive cells among those animals receiving placebo, BZA, and BZA+CEE were comparable and significantly less than the CEE group ($p < 0.05$ for all). On gene microarray analysis, treatment with CEE alone resulted in a greater number of significantly altered genes versus control compared to BZA+CEE and BZA alone ($p < 0.0001$). Principle component and hierarchical clustering analyses revealed that gene expression induced by BZA+CEE more closely resembles BZA than CEE alone. A large group of genes up-regulated by CEE alone were antagonized by the addition of BZA to BZA+CEE co-therapy, but were not significantly regulated by BZA alone. Collectively, these findings indicate that BZA antagonizes the proliferative and transcriptional effects of CEE in estrogen co-therapy while BZA alone has no proliferative effects on the mammary gland in a postmenopausal primate model following 6 months of treatment.

Sources of Research Support: Wyeth (acquired by Pfizer Inc. in October 2009) (an investigator originated grant to TBC); National Institute of Health (NIH) grants, 5T32 RR07009-33 from the National Center for Research Resources (JMC) and R01 AG027847 from the National Institute on Aging (SEA); Intramural grant from the Department of Pathology, Wake Forest University School of Medicine (CEW).

Nothing to Disclose: KFE, CEW, TCR, JMC, SEA, TBC

Pub # P1-17

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Impact of Estradiol Structural Modifications (18-Methyl and/or 17-Hydroxy Inversion of Configuration) on the *In Vitro* and *In Vivo* Estrogenic Activity

Author String DP Ayan, R Maltais, J Roy, D Poirier
Research Center and Faculty of Medicine, Laval University, Québec, Canada

Body It is well recognized that the majority of breast cancers are initially hormone-dependent and that 17 β -estradiol (17 β -E2) plays a crucial role in their development and progression. For this reason, using a compound able to block a specific enzyme involved in the last steps of the biosynthesis of 17 β -E2 remains a rational way to treat estrogen-dependent diseases such as breast cancer. The present study describes the biological *in vitro* and *in vivo* evaluation of three 17 β -E2 isomers. Structural modifications of 17 β -E2 and 17 α -estradiol (17 α -E2) scaffolds at position C18 (inversion of methyl group at position 13 from β to α face, i.e. 18-epi) were achieved. The two isomers 18-epi-17 β -E2 and 18-epi-17 α -E2 were obtained in two chemical steps by inversion of the C18-methyl of estrone using 1,2-phenylenediamine in refluxing acetic acid and reduction of ketone at position C17 with lithium aluminum hydride. The new E2 isomers were tested on estrogen-sensitive cell lines (MCF-7 and T47D) and on estrogen receptor (ER) to determine their estrogenic potency relatively to natural estrogen 17 β -E2. The results show that 18-epi-17 β -E2 possesses the lower affinity for ER (RBA = 1.2 %), the lower estrogenicity on estrogen-sensitive cells (1000 folds less estrogenic than 17 β -E2 in MCF-7) and no uterotrophic (estrogenic) activity when tested on mice. In fact, we observed the following order of estrogenicity: 18-epi-17 β -E2 < 18-epi-17 α -E2 < 17 α -E2 << 17 β -E2. These results suggest that the inversion of C18-methyl of natural 17 β -E2 scaffold could be a useful strategy to decrease the estrogenicity of E2 derivatives used as enzyme inhibitors in the context of a treatment of estrogen-dependent diseases.

Nothing to Disclose: DPA, RM, JR, DP

Pub # P1-18

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title 5 α -Reduced Glucocorticoids as Endogenous Selective Glucocorticoid Receptor Modulators

Author String M Nixon, R Duffin, AG Rossi, BR Walker, R Andrew
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Body Glucocorticoids are converted to 5 α -reduced metabolites, principally in the liver. Recent in vitro data has indicated that 5 α -reduced metabolites of corticosterone (B), namely 5 α -dihydrocorticosterone (5 α DHB) and 5 α -tetrahydrocorticosterone (5 α THB), are glucocorticoid receptor (GR) agonists that exhibit potentially greater effects on inflammatory rather than metabolic pathways. Here, we explored the anti-inflammatory mechanisms of these 5 α -reduced metabolites in vitro and investigated their potential to suppress inflammation in vivo.

In all experiments data are mean % suppression/induction, compared by one way ANOVA, *p<0.05 versus vehicle. In RAW264.7 murine macrophages stimulated with lipopolysaccharide (LPS, 30ng/ml, 24h), TNF α and IL-6 release was suppressed by B (1[μ M]; 61.7%*, 92.2%*, respectively), 5 α DHB (1[μ M]; 20.4%*, 68.8%*) and 5 α THB (1[μ M]; 40.3%*, 47.5%*), effects that were inhibited by co-incubation with GR antagonist RU486 (1[μ M]). Moreover, after 1h incubation with steroids (1[μ M]) prior to LPS stimulation (100ng/ml, 30 min), phosphorylation of kinases p38 and JNK were suppressed by B (42.5%*, 47.5%*, respectively), 5 α DHB (35.7%*, 36.6%*) and 5 α THB (34.9%*, 38.4%*). Protein levels of MKP-1 and I[κ]B α were stimulated by B (160%*, 458%*, respectively) and 5 α DHB (116%*, 389%*), whilst 5 α THB induced MKP-1 (104%*), but not I[κ]B α expression.

For in vivo studies, male mice (C57Bl/6, 8 weeks) received B, 5 α DHB or 5 α THB (250mg/ml, 20[μ l], subcutaneously by injection) or vehicle (DMSO), 2 hours prior to intraperitoneal administration of thioglycollate (10% w/v, 300[μ l]). 4 hours later, peritoneal lavages were performed. Cells were counted and analysed by flow cytometry, and local cytokine levels measured. Total cell infiltration was suppressed by B (45.4%*), 5 α DHB (39.5%*) and 5 α THB (28.8%*). Neutrophil and inflammatory monocyte recruitment was suppressed by B (49.9%*, 96.4%*, respectively), 5 α DHB (55.2%*, 57.3%*) and 5 α THB (58.6%*, 69.5%*). Peritoneal levels of IL-6 and MCP-1 were suppressed by B (86.7%*, 74.0%, respectively) and 5 α DHB (44.9%*, 74.3%*) but unaffected by 5 α THB.

In conclusion, 5 α -reduced corticosterone metabolites exhibit GR-dependent anti-inflammatory properties in vitro in macrophages, with similar efficacy as corticosterone. The ability of these metabolites to suppress inflammation in vivo, together with previous work documenting limited effects on metabolism, demonstrates their potential as an alternative, safer anti-inflammatory steroid therapy.

Disclosures: BRW: Investigator, Wyeth Pharmaceuticals; Ad Hoc Consultant, Boehringer Ingelheim; Astra Zeneca. Nothing to Disclose: MN, RD, AGR, RA

Pub # P1-19

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Compound A Is a Selective Glucocorticoid Receptor Modulator with Potent Anti-Inflammatory Effects and Bone-Sparing Potential

Author String M Rauner, S Thiele, C Goettsch, E Tsourdi, LC Hofbauer
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Body Glucocorticoids (GCs) regulate various physiological processes, including bone remodelling. While physiological amounts of GCs are required for proper human osteoblast differentiation, prolonged exposure to GCs leads to substantial bone loss *in vivo*. Compound A (CpdA) is a novel GC receptor modulator with the potential of an improved benefit/risk profile. In previous studies, we confirmed its anti-inflammatory potential in human monocytes and bone marrow stromal cells and found that -in contrast to dexamethasone- it does not increase the RANKL/OPG ratio. Here, we tested the effects of CpdA on bone metabolism in a mouse model of GC-induced bone loss. Sixty-days release pellets with 2.5, 3.5, and 5 mg/KG prednisolone (PRED) or CpdA were implanted subcutaneously in male FVB/N mice. After 4 weeks, mice were sacrificed and the bone phenotype was assessed. The most profound effects on bone were found in the 5 mg/KG PRED group. At the femur, PRED reduced the total bone density by 10 % ($p < 0.01$), whereas most bone was lost in the subcortical compartment (-12 %, $p < 0.05$), followed by the cortical (-10 %, $p < 0.01$) and the trabecular compartment (-9 %, $p = 0.06$), respectively, as measured by pQCT analysis. At the fourth lumbar spine, PRED induced a 13 % loss of total bone mineral density ($p < 0.01$). The cortical density was reduced by 11 % ($p < 0.01$), the subcortical density by 10 % ($p < 0.05$), and the trabecular density by 13 % ($p < 0.05$). Moreover, serum PINP levels as a marker of bone formation was decreased by 48 % ($p < 0.05$), whereas CTX levels were increased by two-fold ($p < 0.05$). No significant changes were found with any CpdA treatments. Finally, murine bone marrow-derived osteoblasts showed an over 2,000-fold increased RANKL/OPG mRNA ratio when treated with 10 nM PRED ($p < 0.05$), which was not the case for CpdA. Thus, in contrast to PRED, CpdA has no negative impact on bone and does not increase the RANKL/OPG ratio *in vitro*. Whether CpdA also exerts anti-inflammatory effects at this concentration *in vivo* is currently being investigated.

Nothing to Disclose: MR, ST, CG, ET, LCH

Pub # P1-20

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Specificity Conferred upon the Mineralocorticoid Receptor by 11 β -HSD2 Is Effective, but Limited

Author String C Velarde-Miranda, CM Gomez-Sanchez, EP Gomez-Sanchez, CE Gomez-Sanchez
G V Montgomery VA Medical Center, Jackson, MS; University of Mississippi, Jackson, MS

Body The mineralocorticoid receptor (MR) has similar affinity for aldosterone (aldo) and cortisol and corticosteron (cort)(in rodents). Cortisol and cort circulate at concentrations of 1000 (total steroid) and 100 (free)-fold greater than aldo. Specificity of aldo for the MR in transporting epithelia is by co-expression of the 11 β -hydroxysteroid dehydrogenase 2 (11-HSD2) which inactivates glucocorticoids. It has been postulated that 11HSD2 in the kidney is in insufficient quantity to explain MR specificity.
We used a CHO cell infected with lentiviri for the rat MR (pFUGW-rMR), a reporter gene with 3 hormone response elements and the gaussia luciferase reporter (pBM14-TAT3-Gluc), as well as various levels (0, 1, 2 and 3 times) of infection with pCDH-11-HSD2 to increase enzyme expression. The cells released newly synthesized G-luc into the media at 1 hr of incubation with aldo. Studies were done 6 hr after incubation. Aldo and cort were equally potent in producing transactivation of the MR reporter gene in cells that did not express the 11HSD2. In cells infected once with the 11HSD2 virus the kd for stimulation of the reporter gene by aldo was ~ 0.5nM, while that for cort or cortisol was ~0.3 [mu]M. Addition of 1 [mu]M of the 11-HSD2 inhibitor glycyrrhetic acid significantly increased the G-luc production by cort to close of that of aldo. Greater levels of infection with the 11HSD2 lentivirus increased the 11HSD2 mRNA 5-20 fold, but did not change the kd fo transactivation of G-luc reporter gene by cort.
Under non-stressed conditions at the peak of the circadian rhythm the concentrations of total cort are ~ 0.25-0.5 [mu]M and free cort ~ 25-50 nM, concentrations that do not result in transactivation of the MR in these cells. These data suggest that at the normal concentration of cort in blood, 11HSD2 activity is sufficient to block cort transactivation of the MR. At higher than physiological levels of cort (>1 [mu]M), CBG become saturated and the free component increases, the enzyme becomes saturated and cort binds the MR. An increase in the level of expression of the 11HSD2 enzyme does not result in the further elimination of cort within the cell, allowing the remainder to interact with the MR and activate gene transcription. It is likely that NAD⁺, the cofactor for 11HSD2 activity, becomes limiting. Normally intracellular microenvironment where the 11HSD2 and MR reside has low levels of Cort and aldo activates the MR in a specific way.

Sources of Research Support: HL 27255 and the Department of Veterans Affairs.

Nothing to Disclose: CV-M, CMG-S, EPG-S, CEG-S

Pub # P1-21
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)
Title Quantification of 7 α -Hydroxy-4-Cholesten-3-One, a Disease Marker for Cerebrotendinous Xanthomatosis, in Human Plasma Using Novel Derivatization Chemistry and LC-MS/MS
Author String M Weinstock, A DeBarber, B Purkayastha, R Steiner
Oregon Health & Science University, Portland, OR; AB SCIEX, Framingham, MA

Body Introduction:
The ionization efficiency of many neutral steroids or sterols is often not sufficient to reliably quantify low concentrations in small volumes of biological matrix. Derivatization to incorporate an ionizable moiety can significantly improve the ionization efficiency. Described herein a novel method for analysis of the ketosterol 7 α -hydroxy-4-cholesten-3-one (7 α C4) in human plasma. This ketosterol is present at high concentrations in the plasma of patients affected with the neurodegenerative genetic disease Cerebrotendinous Xanthomatosis (CTX). The method utilizes a novel derivatization chemistry to enable sensitive LC-MS/MS analysis of 7 α C4 and can be used for quantification of other ketosterols that are present in CTX, such as 5 α cholestan and 4-cholesten-3-one.

Methods:

Ketosterols in dried blood spots (3[μ L]), were extracted with MeOH+5% acetic acid+ derivatization reagent. The extracts were directly injected into the LC-MS/MS system. Analysis was performed using a Reversed Phase column, ACN/H₂O/Formic acid gradient and API 4000TM MS/MS instrument with ESI source (AB SCIEX). Dominant signature ions specific to each ketosterol were observed in the product ion spectra of the derivatives. Quantification of derivatized 7 α C4 was enabled using isotopically enriched 7 α C4 Internal Standard (IS). A known amount of IS was spiked into calibrants and unknown plasma samples prior to sample work-up and the analyte concentration in unknowns was extrapolated from a calibration curve generated using analyte/IS peak area ratios.

Results:

Upon derivatization, the resultant signal enhancement factor relative to underivatized ketosterol is >270 fold. The limits of Detection for the three CTX biomarkers using 3[μ L] of dried charcoal stripped serum are ~1ng/mL. The sample preparation procedure including the derivatization is simple and amenable to automation. The method provides reproducible and accurate data with good linearity ($R^2 > 0.997$) over a broad dynamic range enabling quantitation of normal healthy controls (6-61ng/mL[2]) and CTX sick samples (>100ng/mL [1]).

Using the above method, 7 α C4 was analyzed in plasma samples of normal and CTX patients. The determined concentrations were in good agreement with the expected values reported in the literature [1,2].

Conclusions:

Derivatization of ketosterol biomarkers with a novel reagent significantly improves the limits of detection by ESI/MS/MS and enables the screening of CTX patients as well as normal healthy controls.

1. DeBarber AE et al., Clin Chim ACTA; 411 (1-2)43-8
2. Camilleri M et al., Neurogastroentrol Motil. 2009;(7):734-e43

Nothing to Disclose: MW, AD, BP, RS

Pub # P1-22

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title The Role of the Estrogen-Related Receptor α in Bone Development and Physiology

Author String MA Dwyer, ER Nelson, X Wang, D Gesty-Palmer, DP McDonnell
Duke University Medical Center, Durham, NC; Duke University Medical Center, Durham, NC

Body The orphan nuclear receptor, Estrogen-Related Receptor α (ERR α), has recently been implicated as a modulator of osteoblast differentiation raising interest in exploring the potential utility of this receptor as a therapeutic target in osteoporosis. However, discrepancies among these previous studies have made it difficult to assess whether ERR α is osteoprotective or if it negatively impacts bone architecture. ERR α knockout mice maintain bone mineral density with age whereas silencing ERR α in primary rat calvaria pre-osteoblasts decreases osteoblast differentiation and mineralization. In this study, we sought to clarify ERR α 's role in bone physiology using a pharmacological approach to antagonize ERR α both *in vitro* and *in vivo*. We observed that treatment of mouse pre-osteoblasts with XCT790, an ERR α antagonist, or ERR α siRNA reduced the mRNA levels of canonical markers of osteoblast differentiation including RUNX2, osteoprotegerin, alkaline phosphatase and osteocalcin. Furthermore, bone marrow derived mesenchymal stem cells exhibited decreased capacity to differentiate into osteoblasts and deposit mineral upon exposure to XCT790. Additionally, the effects of ERR α antagonism on *in vivo* bone parameters were assessed by treating female sham-operated and ovariectomized mice with XCT790 for 28 days using two different dosing regimens. An efficacious dose of XCT790 was achieved as evidenced by changes in the expression of ERR α metabolic target genes in the liver and osteoblast differentiation markers in the calvaria. Serum creatinine and alanine transaminase levels were not significantly elevated in any of the treatment groups indicating no overt toxicity arising from the XCT790 treatment. Bone density analysis by DXA (dual-energy X-ray absorptiometry) indicates ERR α antagonism decreased vertebral spine and mid-femur bone mineral density in both treatment cohorts. Ongoing studies include MicroCT analysis to more precisely evaluate the micro-architecture of these treated animals. Together, our *in vitro* and animal studies indicate that ERR α promotes osteoblast differentiation and bone physiology thus, activation of ERR α may be beneficial for bone maintenance. Furthermore, development of small-molecule agonists for ERR α may provide a novel therapeutic treatment paradigm for osteoporosis or low bone density, pathologies collectively afflicting an estimated 44 million Americans.

Sources of Research Support: D.P.M.: DK048807 & DK074652.

Nothing to Disclose: MAD, ERN, XW, DG-P, DPM

Pub # P1-23

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Osteoclast Progenitors Reside in PPAR γ -Expressing Bone Marrow Cell Population

Author String Y Wan, W Wei, D Zeve, X Wang, Y Du, W Tang, PC Dechow, JM Graff
University of Texas Southwestern Medical Center, Dallas, TX; University of Texas Southwestern Medical Center, Dallas, TX; Baylor College of Dentistry, Texas A & M University Health Sciences Center, Dallas, TX

Body Osteoclasts are bone resorbing cells essential for skeletal development, homeostasis and regeneration. They derive from hematopoietic progenitors in the monocyte/macrophage lineage and differentiate in response to RANKL. However, the precise nature of osteoclast progenitors remains a long-standing yet important question. Using inducible PPAR γ -tTA/TRE-GFP reporter mice, we show that osteoclast progenitors reside specifically in the PPAR γ -expressing bone marrow population, and identify the quiescent PPAR γ ⁺ cells as osteoclast progenitors. Importantly, two PPAR γ -tTA/TRE-Cre controlled genetic models provide compelling functional evidence. First, Notch activation in PPAR γ ⁺ cells causes osteopetrosis due to impaired osteoclast precursor proliferation. Second, selective ablation of PPAR γ ⁺ cells by diphtheria toxin also causes osteopetrosis due to decreased osteoclast number. Furthermore, PPAR γ ⁺ cells respond to both pathological and pharmacological resorption-enhancing stimuli. Mechanistically, PPAR γ promotes osteoclast progenitors by activating GATA2 transcription. These findings not only identify the long-sought-after osteoclast progenitors but also establish unprecedented tools for their visualization, isolation, characterization and genetic manipulation.

Sources of Research Support: University of Texas Southwestern Medical Center Endowed Scholar Startup Fund (Y.W.), a BD Biosciences Research Grant Award (Y.W.), CPRIT grant (RP100841, Y.W.), NIH grants (R01 DK066556, R01 DK064261 and R01 DK088220, J.M.G.), postdoctoral fellowship (W.T.) and predoctoral fellowship (D.Z.) from American Heart Association South Central Affiliate. Y.W. is a Virginia Murchison Linthicum Scholar in Medical Research.

Nothing to Disclose: YW, WW, DZ, XW, YD, WT, PCD, JMG

Pub # P1-24

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Transgenic Skeletal Muscle-Specific Expression of NR4A3/Nor-1 Regulates Several Metabolic Pathways

Author String MA Pearen, NA Eriksson, RL Fitzsimmons, GEO Muscat
The University of Queensland, Brisbane, Australia

Body We have previously reported that both β -adrenergic and α -MSH agonists markedly induced the expression of mRNAs encoding the NR4A subgroup of orphan nuclear receptors (Nur77, Nurr1 and Nor-1) in skeletal muscle (*in vitro* and *in vivo*) (1-2) and adipose tissue respectively (3). We subsequently reported the knockdown of NR4A3/Nor-1 using siRNA in skeletal muscle cells (*in vitro*) and observed decreased palmitate oxidation and increased lactate accumulation consistent with a shift to anaerobic metabolism. Candidate-based mRNA expression profiling indicated that attenuation of Nor-1 altered the expression of genes that activate fatty acid oxidation and aerobic utilization of pyruvate (1). In order to investigate the *in vivo* functional role of Nor-1 expression in skeletal muscle we have now produced a tissue specific transgenic mouse that expresses activated Nor-1 in skeletal muscle. Preliminary analysis of this mouse model suggests that *in vivo* Nor-1 regulates: (i) insulin sensitivity and glucose tolerance, (ii) genes associated with insulin sensitivity, glucose homeostasis and energy usage, and (iii) adiposity.

(1) Pearen MA et al., Endocrinology 2008; 149:2853;

(2) Pearen MA et al., Endocrinology 2006; 147:5217;

(3) Wang SC et al., Mol Endocrinol 2011; Epub

Sources of Research Support: NHMRC Grant #631480 awarded to GEOM.

Nothing to Disclose: MAP, NAE, RLF, GEOM

Pub # P1-25

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Spironolactone Restores Redox Balance during Myocardial Ischemia-Reperfusion

Author String TYL Le, A Ashton, M Mardini, A Mihailidou
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Body **Introduction:** Low doses of mineralocorticoid receptor (MR) antagonists reduce morbidity and mortality in patients with heart failure and myocardial infarction; mechanism(s) currently to be defined. We have recently reported low dose MR antagonists reduce infarct size and apoptosis. Since reactive oxygen species (ROS) generated during reperfusion can lead to myocardial injury and decreased cellular glutathione concentration is an early event in the progression of the apoptotic cascade, we examined whether MR blockade may modify redox state during ischemia-reperfusion (I/R) and how MR antagonists regulate the cardiac-specific anti-apoptotic protein, ARC (Apoptosis Repressor with a Caspase recruitment domain). **Methods:** Adult male Sprague Dawley rats were anesthetised, hearts rapidly excised and subjected to regional ischemia followed by reperfusion ex vivo. MR antagonists were added to perfusates prior to ischemia. The ratio of reduced (GSH) levels and oxidised (GSSG) forms of glutathione was measured using glutathione reductase/5,5'-dithiobis-(2-nitrobenzoic acid) recycling assay and superoxide levels by lucigenin-enhanced chemiluminescence. **Results:** I/R decreased the ratio of reduced [GSH] to oxidised glutathione [GSSG] (1.73 ± 0.31 , N=5 vs 3.79 ± 0.35 (sham), N=5, $p < 0.05$), whereas low dose (10 nM) spironolactone restored redox balance (3.27 ± 0.12 , N=8 vs 3.79 ± 0.35 , N=5). Low dose spironolactone also attenuated IR-induced increased superoxide generation (14.86 ± 1.24 , N=8 vs 21.47 ± 1.25 , N=8, $p < 0.05$). We cultured stable ARC knockdown (siRNA and shRNA) H9c2 cells in which ARC levels were decreased by 70-80%. Hypoxia-induced apoptosis was not prevented by spironolactone, suggesting ARC is required for spironolactone to inhibit apoptosis. **Conclusion:** Our results suggest that MR antagonists restore redox balance during I/R, preventing progression of apoptosis and maintain levels of the anti-apoptotic protein, ARC to protect the myocardium from I/R injury.

Nothing to Disclose: TYLL, AA, MM, AM

Pub # P1-26

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Mineralocorticoid Receptor Antagonism Improves Glomerular and Tubular Proteinuria Independent of Blood Pressure Reduction in Transgenic Model of RAAS Overexpression

Author String A Whaley-Connell, J Habibi, B Krueger, VG DeMarco, L Pulakat, N Rehmer, C Ferrario, JR Sowers
Harry S Truman VA Medical Center, Columbia, MO; University of Missouri Columbia School of Medicine, Columbia, MO; University of Missouri Columbia School of Medicine, Columbia, MO; University of Missouri Columbia School of Medicine, Columbia, MO; Wake Forest University, Winston-Salem, NC

Body
Rational: There is increasing evidence that antagonism of the mineralocorticoid receptor can reduce proteinuria perhaps through improvements in oxidant stress; however, little is known whether this occurs independent of blood pressure. In addition to glomerular origins of proteinuria, recent evidence also suggest impaired tubular reabsorption of protein contributes to low level proteinuria in the face of activation of the renin-angiotensin-aldosterone system (RAAS) and loss of adhesion. Accordingly, this investigation was designed to ascertain if mineralocorticoid receptor antagonism improves glomerular and tubular contributions to proteinuria independent of changes in blood pressure.
Experimental Design: Male TG(mRen2)27 (Ren2) and age-matched Sprague-Dawley rats were treated with either low dose ($\sim 1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) or a vasodilatory, conventional dose ($\sim 30 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) of spironolactone or placebo for three weeks.
Results: Ren2 rats displayed increases in systolic blood pressure (SBP), proteinuria, and the tubular marker beta-N-acetylglucosaminidase (beta-NAG). The Ren2 also displayed increases in the NADPH oxidase subunit Nox2 and a marker for lipid peroxidation 3-nitrotyrosine. Findings that occurred in parallel with reductions in the podocyte specific proteins GLEPP-1, a tyrosine phosphatase, and ezrin, a marker for adhesion, as well as proximal tubule markers Megalin, an endocytotic receptor for albumin, and N-cadherin, a marker for adhesion. Low dose spironolactone had no effect on SBP but improved proteinuria comparable to the conventional dose that led to reductions in SBP. Both doses of spironolactone led to comparable reductions in 3-nitrotyrosine, and increases in GLEPP-1, ezrin, megalin, and N-cadherin.
Conclusions: These data support the notion mineralocorticoid receptor blockade improves glomerular and tubular sources of proteinuria through improvements in oxidative stress and markers of adhesion independent of changes in systolic blood pressure.

Sources of Research Support: VA Career Development Award-2 (AWC), VA Merit Review (JRS), NIH R01 HL73101-01A1 (JRS) and HL-51952 (CMF).

Nothing to Disclose: AW-C, JH, BK, VGD, LP, NR, CF, JRS

Pub # P1-27

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Human Stearoyl-CoA Desaturase-1 (SCD-1) Gene Expression Is Negatively Regulated by Thyroid Hormone in a Sterol-Response Element Binding Protein (SREBP)-1c Independent Manner

Author String K Hashimoto, E Ishida, A Ozawa, N Shibusawa, T Satoh, S Okada, M Yamada, M Mori
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Body Stearoyl-CoA desaturase-1 (SCD-1) plays a pivotal role for an increase of triglyceride by an excess of dietary carbohydrate intake. Dietary carbohydrates increase SCD-1 gene expression in liver by Sterol Response Element Binding Protein (SREBP)-1c dependent and independent pathways. In the SREBP-1c independent pathway, either Carbohydrate Responsive Element Binding Protein (ChREBP) or Liver X receptor (LXR)- α regulates SCD-1 gene expression. It is well known that thyroid hormone gives a great impact for lipid metabolism; however, the molecular role of thyroid hormone in fatty acid synthesis has yet been cleared. Recently, we have reported that thyroid hormone negatively regulates SREBP-1c (1), and positively regulates LXR- α (2) and ChREBP (3) gene expression. In the current study, we examined whether and how thyroid hormone regulates SCD-1 gene expression in liver. SCD-1 mRNA, protein and enzyme activity levels are decreased and increased in thyrotoxic and hypothyroid status in mouse liver, respectively. Luciferase assays using HepG2 cells derived from human hepatocyte revealed that thyroid hormone suppresses both mouse and human SCD-1 gene promoter activity. The responsible region in human SCD-1 gene promoter turned out to be between -124 and -92bp. Chromatin immunoprecipitation (ChIP) assays demonstrated that thyroid hormone receptor (TR)- β but not LXR- α is recruited to the region in a T3 dose-dependent manner. We found a novel negative thyroid hormone response element in the region, which is CGCGGGATGCCGGGC motif. By in silico search, it is revealed that GATA-2 should bind to the motif indicating that GATA-2 could be an important factor for the negative regulation of human SCD-1 gene expression by thyroid hormone. Moreover, the repression by thyroid hormone is independent of SREBP-1c. In conclusion, thyroid hormone negatively regulates both mouse and human SCD-1 gene expression in a SREBP-1c independent manner.

- (1)Hashimoto K et al., Endocrinology 2006;147:4292
- (2)Hashimoto K et al., Endocrinology 2007;148:4667
- (3)Hashimoto K et al., Endocrinology 2009;150:3417

Nothing to Disclose: KH, EI, AO, NS, TS, SO, MY, MM

Pub # P1-28

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Rapid Protein Kinase D1 Signaling Regulates Aldosterone-Mediated ENaC Trafficking in Cortical Collecting Duct Cells

Author String R Dooley, BJ Harvey, W Thomas
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Body Aldosterone stimulates the rapid phosphorylation and activation of Protein Kinase D1 (PKD1) in murine cortical collecting duct cells (M1-CCD), through the transactivation of EGFR (1). The PKD family of serine/threonine kinases are known to be important modulators of subcellular trafficking. PKD1 plays a central role in the regulation of aldosterone-mediated apical trafficking and activity of the epithelial sodium channel (ENaC) in M1-CCD cells. Confocal imaging revealed the aldosterone-mediated insertion of ENaC β to the apical membrane within 24h, whereas in siRNA-mediated PKD1 knockdown cells this effect was abolished. Moreover, aldosterone (10nM) increased the amiloride-sensitive transepithelial current from $(1 \pm 0.21 \text{ [\mu]A/cm}^2)$ to $(7 \pm 0.86 \text{ [\mu]A/cm}^2)$, (n= 8, p < 0.005) in M1-CCD cells within 24h; this effect was significantly reduced in PKD1 knockdown cells (2).
In the distal nephron, ENaC α is under the transcriptional control of aldosterone-bound mineralocorticoid receptor. The Trans Golgi Network (TGN) is the major sorting centre for newly synthesized proteins in the biosynthetic pathway. Using confocal microscopy and immunocytochemistry, we found that aldosterone (10nM) induced the rapid subcellular shuttling of PKD1 from the cytosol to the TGN within 5min of treatment in M1-CCD cells. It was previously shown that PKD1 phosphorylates phosphatidylinositol-4-kinase III β (PI4KIII β) at the Golgi complex to promote vesicle fission (3) (~3 fold as compared to control, n = 6, **p < 0.01). These data begin to unravel the mechanism underlying aldosterone-induced PKD1-mediated trafficking events in the distal nephron.

- (1) McEaney V et al., J Steroid Biochem Mol Biol 2007; 107:180-190
- (2) McEaney V et al., Mol Cell Endocrinol 2010; 325:8-17
- (3) Hausser A et al., Nat Cell Biol 2005; 7:880-886

Sources of Research Support: Higher Education Authority of Ireland under PRTL1 Cycle 4 awarded to BJH; Science Foundation Ireland Research Frontiers Programme grant awarded to WT.

Nothing to Disclose: RD, BJH, WT

Pub # P1-29

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Effect of Steroids on *In Vitro* Melanoma Cell Growth and Viability

Author String P Ramaraj, JL Cox
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Body Skin forms the outer covering of human body. It also protects inner organs from environmental insults such as UV and pathogens. This makes skin vulnerable to various injury and disease. Sex steroids such as testosterone, estrogen, progesterone have roles in maintaining a healthy skin. Any imbalance in these hormones result in skin diseases such as Acne and Alopecia. One of the deadliest form of skin diseases is Melanoma, cancer of the melanocyte. Since there are only few reports on the effect of steroid hormones on melanoma, we decided to check the effect of sex steroids dehydroepiandrosterone (DHEA), Androstenedione (AD), Testosterone (T) and Progesterone (P) on melanoma cell growth and viability.

B16F10 mouse melanoma cells were cultured in 96 well plates and test hormones were added to the growth medium at 50 - 200 [micro]M concentrations for 48 hours and viability was assessed by MTT assay. Of the four steroid hormones tested, Progesterone showed a significant inhibition in cell growth (87% at 200 [micro]M concentration). In order to confirm that it was not a spurious effect of progesterone on melanoma cells, we carried out dose-curve study. It gave a sigmoidal curve with a maximum inhibition of 85% at 150 [micro]M concentration. For specificity sake, we also carried out dose-curve studies with cholesterol, DHEA, RU-486, dexamethasone and estradiol. Cholesterol did not show any significant inhibition of cell growth. DHEA showed a gradual decrease in cell growth up to 42% inhibition at 200 [micro]M concentration. RU-486, an antagonist to progesterone receptor showed a dose-dependent inhibition of cell growth with a maximum inhibition of 89% at 200 [micro]M concentration. Dexamethasone, an agonist of glucocorticoid receptor and estradiol did not show significant inhibition. Of all the steroids tested in the dose-curve experiments, only Progesterone and RU-486 showed significant inhibition of B16 cell growth (42% at 50 [micro]M concentration of progesterone and 68% at 50 [micro]M concentration of RU-486). The same pattern of inhibition was observed with these two steroids on metastatic BLM human melanoma cell line (53% at 50 [micro]M concentration of progesterone and 76% at 50 [micro]M concentration of RU-486). Thus, progesterone and RU-486 showed dose-dependent inhibition of melanoma cell growth *in-vitro* both in murine and human cell lines. Progesterone and RU-486 treatment not only decreased cell growth, but also resulted in cell death. RU-486 was more inhibitory to melanoma cell growth than progesterone.

Nothing to Disclose: PR, JLC

Pub # P1-30

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title SLIRP, a Nuclear Receptor Coregulator, Is a Good Prognostic Factor in Colorectal Cancer

Author String PA Candy, SM Colley, M Phillips, A Davidson, B Iacopetta, L Stuart, B Wood, A Redfern, PJ Leedman
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Body Colorectal cancer (CRC) is the second leading source of cancer morbidity in the Western world, accounting for 52,000 deaths in the United States per annum. Key pathways that drive CRC growth include the Notch and beta-catenin networks. Although a variety of nuclear receptors (NRs) have been implicated in CRC pathogenesis and response to treatment, little is known of the role of NR coregulators in this disease. Previously we discovered SLIRP, a Steroid receptor RNA Activator (SRA)-binding NR coregulator, which is a potent repressor of NR transcriptional activity. We have also generated data in other systems showing that SLIRP can regulate the Notch signaling pathway. Consequently, in these studies we investigated the expression of SLIRP in human CRC and its potential to modulate NR and Notch signaling. Using a human CRC tissue microarray cohort of 1044 patients (Stage 2 and 3 tumors) and four independent mRNA CRC patient microarray databases, we found that elevated SLIRP expression was significantly associated with a higher 5-year survival and lower relapse rate ($p < 0.001$). In addition, SLIRP expression was strongly inversely associated with tumor stage and lymph node invasion. When SLIRP expression in human CRC cells was reduced with siRNA invasion (using matrigel assays) and Notch signaling (using a HES-1 luciferase reporter) both were significantly increased. Furthermore, CRC cells treated with SLIRP siRNA were more resistant in growth assays to the baseline CRC chemotherapeutic agent, 5-Fluorouracil (5-FU). Taken together, these data provide the first evidence that SLIRP is a good prognostic factor in CRC and suggest that this protective effect may in part be due to suppression of Notch signalling, reduced invasion and enhanced chemotherapeutic sensitivity.

Sources of Research Support: NHMRC, RPH Medical Research Foundation and Cancer Council of WA.

Nothing to Disclose: PAC, SMC, MP, AD, BI, LS, BW, AR, PJJ

Pub # P1-31

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Loss of the Nuclear Receptor Corepressor SLIRP Compromises Male Fertility *In Vivo*

Author String SM Colley, R Searles, L Wintle, V Russell, RC Firman, S Smith, K Deboer, B Genevieve, B Stuckey, LW Simmons, M O'Bryan, BW O'Malley, PJ Leedman
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Body Nuclear receptors (NRs) and their coregulators play fundamental roles in initiating and directing gene expression influencing mammalian reproduction, development and metabolism. SRA stem Loop Interacting RNA-binding Protein (SLIRP) is a Steroid receptor RNA Activator (SRA) RNA binding protein which is a potent repressor of NR activity. SLIRP is present in NR complexes associated with NR target genes in the nucleus, however, it is also abundant in mitochondria where it may affect energy turnover. Notably, SLIRP is a potent regulator of androgen action and is expressed in the testis and more specifically within developing spermatozoa. Colocalization studies indicate SLIRP is present within the mid-piece and head of the mature sperm suggesting a functional role within this tissue. To investigate the *in vivo* effects of SLIRP, we have generated a SLIRP knock out (KO) mouse. This animal is viable, however when homozygous males are crossed with wild type (wt) or heterozygous females the resultant litter size is reduced by approximately one quarter (-/- x +/+, 4.8 pups/litter; +/- x +/-, 6.3) compared with those produced by wt males with comparable females (+/+ x +/+, 6.6; +/+ x +/-, 6.7). Comparison of sperm samples from males of each of the phenotypes showed that the KO mice had 60% fewer progressively motile sperm than either the wt or homozygous mice. In humans, micro-array data suggests that SLIRP mRNA levels in sperm from teratozoospermic men are frequently reduced. In sum, our data suggests that loss of SLIRP results in impaired male fertility, attributable in part, to compromised sperm motility.

Nothing to Disclose: SMC, RS, LW, VR, RCF, SS, KD, BG, BS, LWS, MO, BWO, PJJ

Pub # P1-32

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title A Peroxisome Proliferator Activated Receptor γ (PPAR γ) Coactivator 1 β Autoregulatory Loop in Adipocyte Mitochondrial Biogenesis

Author String T Deng, D Sieglaff, A Zhang, C Lyon, S Ayers, A Cvaro, X Xia, JD Baxter, P Webb, WA Hsueh
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Body Thiazolidinediones (TZDs) act through peroxisome proliferator activated receptor γ (PPAR γ) to induce adipogenesis and to enhance mitochondrial biogenesis in the adipocyte. Whereas previous studies have suggested a role for PPAR γ coactivator 1 α (PGC-1 α) in the latter effect, mechanisms involved in TZD-dependent regulation of adipocyte mitochondrial content and activity are not clear. It was recently shown that PPAR γ induces PGC-1 β in osteoclasts to initiate a transcriptional feed-forward loop required for TZD-dependent gene regulation. Here, we show that TZDs also act through PPAR γ to induce PGC-1 β in cultured 3T3-L1 adipocytes. This effect, however, is rapid, direct and involves PPAR γ interactions with a hitherto unknown intronic PPAR γ response element (PPRE) cluster in the PGC-1 β locus, different from the indirect mechanism proposed to explain TZD induction of PGC1- β in osteoclasts. Fractionation of obese mouse adipose tissue reveals that PPAR γ and PGC-1 β , but not PGC-1 α , are coordinately upregulated during pre-adipocyte to adipocyte differentiation. Further, we find that TZDs upregulate PGC-1 β and mitochondrial marker genes in white adipose tissue (WAT) depots of different obese mouse models. Use of short interfering RNA confirms that TZD- induction of mitochondrial genes and mitochondrial activity in 3T3-L1 cells is dependent on PGC-1 β . We propose that PPAR γ directly induces PGC-1 β expression in adipocytes and that this effect plays a special role in regulation of mitochondrial biogenesis and activity in this cell type.

Sources of Research Support: MacDonald Foundation; American Diabetes Association Fellowship.

Nothing to Disclose: TD, DS, AZ, CL, SA, AC, XX, JDB, PW, WAH

Pub # P1-33
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)
Title Role of Corepressor Complex in Modulating PPAR γ Function in Mature Adipocytes
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Body The peroxisome proliferator-activated receptor γ (PPAR γ) is a nuclear receptor and the key protein controlling adipocyte function. It is also the cellular target of insulin-sensitizing drug thiazolidinediones (TZDs), the synthetic agonists of PPAR γ used in treating diabetic patients. However, side effects of TZD treatment, mostly coming from full agonist activity of PPAR γ , largely limit the effectiveness for the patients. As a transcriptional factor, PPAR γ is closely regulated by coregulators, which include coactivators and corepressors. Recent studies have revealed that in macrophages, insulin-sensitizing effect of PPAR γ may involve suppression of proinflammatory gene expression by recruitment of the corepressor complex that contains corepressors and histone deacetylases (HDACs). However, the role of corepressor complex in adipocytes remains largely unknown. To investigate the role of the corepressor complex in modulating adipocyte function, we used RNA interference to deplete PPAR γ corepressor (the silencing mediator of retinoic acid and thyroid hormone receptor, SMRT), or applied HDAC inhibitor trichostatin A (TSA) in 3T3-L1 adipocytes. The corepressor complex, including SMRT and HDAC activity, appeared to involve in insulin-stimulated glucose uptake in adipocytes, since SMRT depletion and TSA treatment both reduced insulin-stimulated glucose uptake and insulin-stimulated translocation of glucose transporter 4 (GLUT4). Insulin signaling through insulin receptor substrate (IRS) and Akt remained intact in either SMRT-depleted or TSA-treated adipocytes, whereas insulin-stimulated tyrosine phosphorylation of Cbl was attenuated in either condition. Moreover, TZD-enhancement on insulin-stimulated glucose uptake was attenuated in either SMRT depleted or TSA-treated adipocytes, suggesting overlapping mechanism mediated by both SMRT and HDACs in insulin-stimulated glucose uptake in adipocytes. In contrast, HDAC activity, but not corepressor NCoR/SMRT, was required for TZD suppression of TNF α -induced proinflammatory gene expression in adipocytes. These results highlight differential requirement of the corepressor complex in modulating PPAR γ function in adipocytes.

Nothing to Disclose: J-CL, TI, Y-TC, Y-CL, JMO

Pub # P1-34

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title UDCA Inhibits Nuclear Receptor LXR α Transactivation through Induction of Transcriptional Corepressor SMILE

Author String J-M Lee, H-S Choi
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Body SMILE (small heterodimer partner interacting leucine zipper protein) has been identified as a nuclear corepressor of nuclear receptors (NRs) family. Here, we examined the role of SMILE on the regulation of nuclear receptor LXR α (liver X receptor α). Transient transfection assays demonstrated that SMILE represses LXR α agonist (T7 (TO901317))-induced LXR α transactivation and *SREBP-1c* gene promoter activity. Overexpression of SMILE by adenovirus SMILE inhibits T7-induced mRNA levels of LXR α target gene *SREBP-1c*. *In vivo* and *In vitro* glutathione S-transferase pull-down assays revealed that SMILE physically interacts with LXR α . Furthermore, to determine whether UDCA-mediated inhibition of LXR α transactivation and LXR α target gene expression depend on SMILE gene expression, we measured the effect of UDCA on *SREBP-1c* gene expression when knockdown of endogenous SMILE gene expression. UDCA (ursodeoxycholic acid) increases SMILE promoter activity and mRNA levels in time and dose dependent manners. UDCA repressed T7-induced *SREBP-1c* gene promoter activity and protein levels, whereas knockdown of endogenous SMILE expression by adenovirus SMILEshRNA significantly reversed UDCA-mediated inhibition of *SREBP-1c* protein levels. Collectively, these results demonstrated that UDCA-induced SMILE gene expression inhibits LXR α transactivation and LXR α target gene expression.

Nothing to Disclose: J-ML, H-SC

Pub # P1-35

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title Dysregulation of a SMRT/GPS2 Corepressor Complex Is Associated with Adipose Tissue Inflammation in Human Obesity

Author String N Venteclef, R Allili, E Treuter, K Clement
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Body **INTRODUCTION:** Obesity has become a global epidemic and is one the most significant public health challenges of the 21st century. In recent years, evidence has emerged that inflammation is the crucial physiological process behind obesity complications. Studies from our group have reported how deregulation of adipose tissue secretory functions contributes to obesity complications. However, the precise molecular mechanisms that integrate the inflammatory response with metabolic homeostasis at the cellular and systemic level remain unclear. Based on previous implications (1) we investigated here the role of the NCOR/SMRT/GPS2 corepressor complex as a transcriptional key component of obesity-induced inflammation in human adipose tissue.
METHODS: Gene expression and protein recruitment onto inflammatory gene promoters of corepressor complex components were analyzed in human adipose tissue. In addition, siRNA experiments have been performed in human pre-adipocytes to characterize the biological function of the complex.
RESULTS: Individual subunits of the corepressor complex appear differentially regulated in adipose tissue of non-obese versus obese subjects. Expression level of the corepressor complex is associated with the adipose tissue inflammatory status of obese subjects. siRNA experiments reveal the importance of the complex in the control of adipose cell inflammation. Taken together, the data suggest that targeting the co-repressor complex could be an alternative strategy to control inflammatory processes linked to obesity.
DISCUSSION: Our study extends the knowledge of anti-inflammatory mechanisms directed by the GPS2-containing corepressor complex and implies alternative pharmacologic strategies for the treatment of obesity complications.

(1) Venteclef N et al. Genes Dev 24, 381-395, 2010

Nothing to Disclose: NV, RA, ET, KC

Pub # P1-36

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Biological Effects of Nuclear Receptors, Coregulators & Ligands (1:30 PM-3:30 PM)

Title The Nuclear Corepressor, NCoR, Controls Cholesterol Metabolism in the Liver

Author String I Astapova, KA Holtz, AN Hollenberg
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Body NCoR has been proposed to play an important role in nuclear receptor (NR) signaling by mediating transcriptional repression by NRs. However, its in vivo function remains poorly understood as global deletion of NCoR is embryonic lethal. We have created a mouse model that can conditionally express a mutant NCoR (NCoR[Delta]ID) protein, that is defective in its ability to interact with NRs. Using a liver-specific mutant (L-NCoR[Delta]ID) we have previously demonstrated that NCoR mediates repression of positively regulated T3 target genes by the unliganded thyroid hormone receptor in the hypothyroid state and also attenuates T3 signaling in the euthyroid state. Surprisingly, while mRNA expression of the rate-limiting enzyme of cholesterol conversion to bile, Cyp7a, was significantly upregulated in L-NCoR[Delta]ID mice, this did not prevent the increase in serum cholesterol seen in hypothyroidism. Analysis of hepatic gene expression pattern in euthyroid and hypothyroid mice revealed that expression of a number of genes in the cholesterol biosynthesis pathway were increased in L-NCoR[Delta]ID as compared to controls potentially explaining unaffected serum cholesterol levels. This observation together with the fact that NCoR[Delta]ID has reduced capacity to recruit liver X receptor α (LXR α), the key regulator of cholesterol metabolism, prompted us to study cholesterol metabolism in L-NCoR[Delta]ID mice in more detail. Remarkably, after 3 weeks on a high cholesterol diet, L-NCoR[Delta]ID animals have decreased cholesterol deposition in the liver compared to wild type controls (4.5 mg/g vs 13.5 mg/g). This was accompanied by elevated serum triglyceride concentrations due to an increase in VLDL production, but normal serum cholesterol and hepatic triglyceride levels. While these findings might indicate augmented LXR signaling, we found no difference in the mRNA expression levels of LXR target genes implicated in cholesterol catabolism and clearance such as Cyp7a and Abcg5/Abcg8. However, mRNA expression of the bile salt export pump (BSEP) a key regulator of bile acid excretion and a FXR-target was upregulated in L-NCoR[Delta]ID animals, suggesting that this might be one of the molecular determinants of enhanced cholesterol conversion and clearance. While the exact mechanism remains to be elucidated, these data indicate that NCoR plays an important role in cholesterol metabolism in the liver, and interactions between NCoR and NRs may represent a new therapeutic target for reducing cholesterol levels.

Sources of Research Support: NIH NIDDK DK-056123.

Nothing to Disclose: IA, KAH, ANH

Pub # P1-37

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Development & Epigenetics of Steroid Biology & Neoplasia (1:30 PM-3:30 PM)

Title Overexpression of the Hedgehog Pathway in Potential Adrenal Progenitor Cells

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Body The Hedgehog signaling pathway has been implicated in a variety of developmental processes including cell fate, proliferation and survival together with organ patterning and homeostasis. Intracellular signaling is initiated by binding of secreted ligands, Sonic hedgehog (Shh), Indian Hedgehog (Ihh) or Desert hedgehog (Dhh) to the cell surface receptor Smoothed (Smo). In absence of ligand, Smo is inhibited by another membrane protein, Patched (Ptch). Upon ligand binding Smo is released from Ptch and activates nuclear translocation of the transcription factors Gli1, Gli2, and Gli3. In the adrenal gland, Gli1 and Ptch are expressed primarily in the capsule while the predominant ligand, Shh, is found in the subcapsular region of the cortex.

Hedgehog signaling has been shown to be required for adrenal organogenesis. In recent studies, genetic loss of Shh in the adrenal cortex of tissue-specific Shh knockout mice resulted in reduced proliferation of capsular cells and a reduction in both capsule thickness and adrenal size. Moreover, lineage tracing studies demonstrated that Gli1-positive, Shh responsive, cells are organized in clusters in the adrenal capsule and can migrate centripetally into the cortex giving rise to differentiated cells of the adrenal cortex (1,2). Data from several studies have defined a complex interplay between the Hedgehog pathway and the Wnt pathway, another signaling pathway critical to adrenal development and implicated in adrenocortical tumor formation (3). While Shh-responsive cells reside in the adrenal capsule, Wnt-responsive cells are restricted to the subcapsular cortex, predicting a potential complimentary regulatory system.

To further investigate the role of the Shh pathway in adult adrenocortical homeostasis, we have generated a mouse line in which constitutively active Smo is inducibly expressed under control of the Gli1 promoter. Overexpressing Smo in these Gli1-positive capsular cells results in an unexpected thinning of capsular (COUPTFII-positive) cells and an increase in subcapsular proliferation that is coincident with enhanced staining of β -catenin and glomerulosa restricted Cyp11 β expression. Current efforts explore the molecular underpinning of the interaction between the Wnt and Shh pathway in the adrenocortical development and homeostatic maintenance.

(1) King P et al., Proc Natl Acad Sci U S A (2009); 106(50): 21185-21190
(2) Huang CC et al., Endocrinology (2010); 151(3): 1119-1128
(3) Kim AC et al., Development (2008); 135(15): 2593-2602

Disclosures: GDH: Ad Hoc Consultant & Study Investigator, OSI; Ad Hoc Consultant, Orphagen, HRA Pharma; Study Investigator, Corcept. Nothing to Disclose: IF

Pub # P1-38

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Development & Epigenetics of Steroid Biology & Neoplasia (1:30 PM-3:30 PM)

Title Multigenerational Programming: Generation-Specific and Parent-of-Origin Effects

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Body Exposure to an adverse environment in-utero is linked to cardiometabolic disease 'programming'. In a rat model of prenatal glucocorticoid overexposure we showed programmed effects on birthweight and glucose tolerance were recapitulated in a second generation. Given the phenotypic similarity, we hypothesized changes in the expression of genes regulating fetoplacental growth and nutrient transport, key programming targets, would be similar in both generations.

Female Wistar rats received Dexamethasone (Dex) or Vehicle (Veh) during the last week of gestation (E15-E21). Females were either culled at E20 for fetal/placental collection or delivered F1 offspring. F1 offspring were mated in all combinations to give four F2 groups. Tissues were collected at E20. RNA was extracted from placenta and fetal liver and gene expression studied by real-time PCR.

Dex exposure reduced F1 placental (Veh 0.44 ± 0.01 vs Dex 0.38 ± 0.01 g; $p < 0.001$) and fetal (Veh 2.38 ± 0.03 vs Dex 2.15 ± 0.04 g; $p < 0.001$) weight. In F1 Dex placentas, consistent with reduced placental size, expression of the prenatal growth factor Igf2 was decreased whilst expression of nutrient transporters was increased. Although Dex exposure increased fetal liver Igf2 expression, expression of the growth restricting gene Cdkn1c was also increased. In contrast, in the F2 generation there were marked parent-of-origin effects on fetoplacental growth and gene expression: maternal prenatal Dex increased placental ($F=10.15$, $p < 0.01$) and fetal ($F=17.69$, $p < 0.001$) weight and placental expression of growth promoting genes (Mest, Slc38a4 and Glut1) whereas paternal prenatal Dex decreased placental ($F=12.25$, $p < 0.001$) and fetal ($F=4.78$, $p=0.029$) weight and increased placental expression of growth restricting genes (Cdkn1c, Phlda2). In F2 fetuses, there was an interaction between parental Dex exposure to reduce Igf2 ($F=5.66$, $p=0.025$) and Phlda2 ($F=6.62$, $p=0.016$) expression.

Thus, the multigenerational effects of glucocorticoid programming involve distinct processes in F1 and F2 despite phenotypic similarities. Direct Dex exposure has multiple, tissue-specific effects on gene expression which may interact to reduce fetoplacental growth. In contrast, effects on fetal and placental growth differed in the F2 generation and were highly dependent on parental prenatal Dex exposure. These findings have implications for the pathogenesis and future attempts to stratify therapies for the 'developmental component' of cardiometabolic disease.

Nothing to Disclose: LL, JRS, AJD

Pub # P1-39

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Development & Epigenetics of Steroid Biology & Neoplasia (1:30 PM-3:30 PM)

Title P53 and CTNNB1 Mutations Reveal Different Patterns between Pediatric and Adult Adrenocortical Tumors

Author String LM Mermejo, LF Leal, LM Colli, CE Martinelli, AC Moreira, LG Tone, CA Scrideli, JA Yunes, MJ Mastellaro, AL Seidinger, SR Brandalise, LN Ramalho, SR Antonini, M Castro
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Body **Introduction:** Somatic inactivating mutations of the tumor suppressor gene *TP53* and activating mutations of the protooncogene beta-catenin (*CTNNB1*) are the most frequent mutations identified in adrenocortical tumor: (ACTs). **Objective:** To analyze the R337H p53 and *CTNNB1* gene mutations in children and adults with ACTs and verify the association of mutations in these genes with tumor stage and mortality. **Patients and Methods:** We studied 71 patients with ACT, 62 children (48F/14M; median age: 1.8 yrs, range: 0.4-15.5) and 9 adults (8F/1M; median age: 39.6 yrs, range: 27-55). Children presented hormone excess: 34 virilization (V), 26 virilization and Cushing's syndrome (V+C), 2 Cushing's syndrome (C). Based on Sandrini classification, 37 patients presented stage I, 9/II, 8/III and 8/IV. Median follow-up was 6.6 yrs, 11/59 patients (18%) died and 3 lost follow up. Regarding adult clinical presentation, we observed 3V+C, 3C, 1V and 2 non-secreting tumors. Based on Macfarlane classification, 4 patients presented stage I, 2/II, 1/III and 2/IV. Median follow-up was 3.8 yrs, 2/9 patients (22%) died. Exon 10 of the *TP53* and exon 3 of *CTNNB1* genes were amplified by PCR and sequenced by automatic sequencing. Immunohistochemical analysis was performed in a subset of 22 children and 5 adult ACTs using beta-catenin and P53 monoclonal primary antibodies. **Results:** The R337H p53 mutation was found in a higher incidence ($p<0.0001$) in children (56/62, 90%) compared to adults (2/9, 22%). On the other hand, *CTNNB1* mutations were found in a higher incidence ($p<0.001$) in adults (5/9, 55%) compared to children (4/62, 6%). Among these *CTNNB1* mutations, we found the deletion p.S45_G50del, which has not yet been described. Moderate and/or strong beta-catenin and P53 cytoplasmatic and/or nuclear accumulation were observed in 15 (68%) and 9 (40%) out of 22 children ACTs, respectively, and in all five adult ACT samples. There was no association of *TP53* or *CTNNB1* mutation and tumor stage as well as with higher mortality in adults, however the presence of *CTNNB1* mutation was associated with increased mortality in children ($p=0.02$). **Conclusion:** Whereas R337Hp53 germline mutation is found in most of Brazilian children ACTs, *CTNNB1* gene mutations are more frequent in adult Brazilian ACTs. It is important to point out that *CTNNB1* mutations were found in children and adult ACTs harboring *P53* mutation, indicating that these mutations are not mutually exclusive, as it has been recently suggested.

Sources of Research Support: Fapesp 2008/09276-0.

Nothing to Disclose: LMM, LFL, LMC, CEM, ACM, LGT, CAS, JAY, MJM, ALS, SRB, LNR, SRA, MC

Pub # P1-40

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Development & Epigenetics of Steroid Biology & Neoplasia (1:30 PM-3:30 PM)

Title Proliferative Effect of 1-28 N-POMC on Rat Adrenal Medulla and Cyclin D Protein Expression in Adrenal Cortex

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Body A previous work from our group (1) showed that the 1-28 N-terminal peptide of pro-opiomelanocortin (N-POMC), with (NP^W) or without (NP^{W/O}) disulfide bridges, promotes an increase in BrdU positive cells in the adrenal cortex. Moreover, cyclin E appears to be involved in the N-POMC- and ACTH-stimulated proliferation in the Zona Glomerulosa (ZG), but not in Zona Fasciculata (ZF) and Zona Reticularis (ZR) of the adrenal cortex. Cyclins D and E are the major cell cycle controls in G1[rarr]S transition, but in the adrenal cortex the effect of ACTH and N-POMC on the expression of these proteins remains incipient. By using NP^W and NP^{W/O} (methionine replacing cysteines), we evaluated the importance of disulfide bridges in the proliferative response on adrenal medulla, and the effect of ACTH and 1-28 N-POMC on cyclins D proteins in rat adrenal cortex. Sprague Dawley male rats were treated with dexamethasone (DEX) (50 [micro]g/100 g B.W.), and injected with ACTH (A) (100 [micro]l 10⁻⁷M /100 g B.W.) and/or NP^{W/O} or NP^W (100 [micro]l 10⁻⁷M /100 g B.W.). DNA synthesis was evaluated by BrdU (100 [micro]g/100 g B.W.) incorporation and protein expression by immunoblotting (IB) by using protein extract of external (EF: capsule+ZG) and internal (IF: ZF+ZR without Medulla) fraction from adrenal cortex. Injections with NP^W, NP^{W/O} and A+NP promoted a significant BrdU-positive nuclei increase in chromaffin cells, in contrast with treatment with ACTH. By using IB, in the EF, neither cyclin D1 nor D2 were modulated after treatments, whereas D3 was significantly down regulated after 6h-DEX and A+NP^{W/O} (0.65 and 0.56 fold). In the IF D1 also was not regulated in contrast with D2 that was up regulated with 4h-A+NP^{W/O} (3 fold). D3 expression was increased after A, NP^W and A+NP^{W/O} at 4h (1.4, 1.4 and 1.6 fold) and sustained by 6h. In summary, cyclin D3 seems to be involved with the A and NP proliferative induction in the inner cortex, whereas the increase of BrdU-positive nuclei suggests a mitogenic effect of N-POMC peptides in medulla, regardless of disulfide bridges presence.

(1) Mendonca,P.O.R.d.,Lotfi,C.F.P.,Mol Cell Endocrinol 2011; doi:10.1016/j.mce.2010.12.012

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Nothing to Disclose: PORdM, RBT, CFPL

Pub # P1-41

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Development & Epigenetics of Steroid Biology & Neoplasia (1:30 PM-3:30 PM)

Title Phosphodiesterase 11A (Pde11a) Expression in Mouse Tissues and Characterization of a Pde11a Mouse Knock-Out Model

Author String I Levy, M Starost, E Ball, FR Faucz, S Koliavasillis, A Horvath, K Tsang, K Nadella, M Alatsatianos, M Nesterova, CA Stratakis
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Body Phosphodiesterases catalyze the hydrolysis of cyclic nucleotides and maintain physiologic levels of intracellular concentrations of cAMP and cGMP. Increased cAMP signaling has been associated with genetic disorders that lead to adrenocortical tumors and Cushing syndrome.¹ Genetics defects in the phosphodiesterase 11A (PDE11A) are associated with increased levels of cAMP and bilateral adrenal hyperplasia;² they also contribute to the development of adrenocortical, prostate, and testicular tumors in the general population.³ The aim of the present work is to study the expression of Pde11a in mouse tissues, as well as to understand better the Pde11a defect in a previously published Pde11a^{-/-} knockout(KO) mouse model.⁴ A colony of Pde11a^{-/-}(KO), Pde11a^{+/-}(Het) and wild type(wt) mice was studied in a phenotypical gross and microscopy pathological study. Molecular, immunohistochemical and biochemical studies were done to determine the function and expression of Pde11a in the tissues of normal and mutant mice. In normal mice higher PDE11A immunoreactivity(PDE11A-IR) was found in small intestine villi, thyroid follicular cells, prostate and spermatocytes; moderate PDE11A-IR in brain, liver hepatocytes, and exocrine pancreas and little, if any, in the pituitary gland. In mutant mice RNA studies showed a reduced Pde11a expression in steroidogenic tissues (adrenals and testis), lung, brain, and liver while prostate and kidney presented comparable expression with normal mice. PDE11A protein expression and activity assays did not show differences among three groups. Pathological studies showed differences: Adrenal subcapsular hyperplasia and foamy cells were more frequent in Het(37.7% and 30.2% respectively, p<0.05) than in wt group (26.3% and 15.8% respectively); Spinal epidermoid cyst in Het (15%) and wt mice (10%); kidney-pelvis interstitial lymphocytes only in KO mice (10.7%, P<0.05). KO mice showed increased absolute and organ specific weight. This study show a clear profile of Pde11a protein expression in endocrine and other mouse tissues, as well as convincing evidence of only partial Pde11a suppression in the previously published KO mouse. These mice developed adrenal hyperplasia and other abnormalities like humans with heterozygote PDE11A defects and partial inactivation of the enzyme. These data support the involvement of PDE11A in the pathogenesis of adrenocortical hyperplasia, but also provide significant insight into a previously reported mouse model.

(1) Almeida M. et al. Mol Cell Endocrinol. 2010 Nov 25

(2) Horvath et al. Nat Genet. 2006 Jul;38(7):794-800.

(3) Horvath et al. Cancer Res. 2009 Jul 1;69(13):5301-6.

(4) Wayman C. Int J Impot Res. 2005;17:216

Nothing to Disclose: IL, MS, EB, FRF, SK, AH, KT, KN, MA, MN, CAS

Pub # P1-42

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Development & Epigenetics of Steroid Biology & Neoplasia (1:30 PM-3:30 PM)

Title Targeted Inhibition of c-KIT Activation Sensitizes Adrenocortical Tumor Cells to Apoptosis

Author String KS Nadella, M Nesterova, M Lodish, E Ball, P Xekouki, E Saloustros, C Stratakis
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Body Primary Pigmented Nodular Adrenocortical Disease (PPNAD), a type of micro nodular adrenal hyperplasia (MAH) that occurs mostly in the context of Carney Complex and rarely in an isolated form is caused by germline mutations in *PRKARIA*, the main regulatory subunit of PKA. In addition, defects in Phosphodiesterase genes 11A (*PDE11A*), 8B (*PDE8B*) and abnormalities of cAMP signaling are known to occur in a small fraction of MAH and massive macronodular adrenocortical disease (MMAD) respectively, suggesting that dysregulation of PKA is a key player in the genesis of these lesions. The only treatment option is adrenalectomy resulting in adrenal insufficiency. In search of therapeutic targets to develop alternative strategies to treat this disease, we identified for the first time tumor specific over expression and constitutive activation of c-Kit in PPNAD compared to cortisol-producing adenomas (CPA), MMAD and aldosterone producing adenomas (APA). We investigated the mutational status of the signature exons in *c-KIT* gene and found no mutations. Real-time PCR analysis confirmed upregulation of c-Kit. c-Kit overexpression was recapitulated in adrenals from *Prkar1a* knockout mice reminiscent of that seen in human patients suggesting PKA specific dysregulation of c-Kit expression. To better characterize these changes at the biochemical level we used an adrenocortical cell line (CAR 47.01) derived from a patient with mutant PRKAR1A and compare it to the H295R adrenocortical cancer line. Consistent with tumors, CAR47.01 cells exhibited enhanced activation of c-Kit which was further enhanced by treatment with forskolin, a PKA activator. This upregulation was markedly rescued by reintroduction of PRKAR1A suggesting that PKA functions upstream of c-Kit. *In vitro* kinase assay demonstrated c-Kit to be a direct phosphorylation target of PKA. To explore whether overexpressed c-Kit is a therapeutic target, the cells were treated with imatinibmesylate which resulted in reduced cell viability and induced growth arrest and apoptosis in a time- and dose dependent manner. In the CAR47.01 cells imatinib treatment induced downregulation of phospho-Kit and phospho-Akt, an immediate downstream phosphorylation target of c-Kit without altering total c-Kit and Akt levels. Taken together, our data provide novel insights on PKA specific regulation of c-Kit and indicate c-Kit as a promising potential target for therapeutic intervention in adrenocortical neoplasms.

Nothing to Disclose: KSN, MN, ML, EB, PX, ES, CS

Pub # P1-43

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Development & Epigenetics of Steroid Biology & Neoplasia (1:30 PM-3:30 PM)

Title Different Expression Patterns for MRAP α and MRAP2 mRNA in Mouse Adrenal Gland

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Body MRAP α was the first melanocortin 2 receptor (MC2R) accessory protein discovered and its presence is critical for the MC2R to functionally express either *in vivo* or *in vitro* (1). MRAP2 is a homologous MRAP α gene and while it can enable the MC2R to functionally express *in vitro*, it does not allow MC2R to functionally express *in vivo*. To further understand physiological roles for MRAP α and MRAP2, we have mapped mRNA expression for these genes in adrenal glands dissected from male C57BL/6J mice aged 2-150 days. We developed riboprobes to specifically detect either mouse MRAP α or mouse MRAP2. Antisense riboprobes synthesized with [³³P]-UTP were used for high stringency *in situ* hybridization performed on three series of 20[μ] adrenal sections that had been cut on a cryostat after adrenal glands were fixed with 4% paraformaldehyde and frozen in embedding media. One series was used for *in situ* hybridization performed with a [³³P]-labelled antisense mouse MC2R specific riboprobe. Adrenal glands were collected from at least 2 mice derived from different parents, and they were aged 2, 4, 7, 10, 15, 21, 30, 45, 60, 100 or 150 days. MRAP α was detected throughout the adrenal cortex from 2-10 day old mice and then predominantly in the zona fasciculata (ZF) region in mice aged 15-150 days. MRAP α expression was also present in the X-zone adjacent to the medulla, with strongest expression seen around puberty. MRAP α expression was very weak in the zona glomerulosa (ZG) and not expressed at all in the adrenal medulla. In contrast to MRAP α , MRAP2 expression was strongest in the ZG and adrenal medulla in tissues collected from mice aged 15-150 days. There was strong MRAP2 expression over the entire adrenal gland obtained from 2-10 day old mice. MC2R mRNA was expressed throughout the adrenal cortex at all ages, predominantly in the ZF from 10-21 days and predominantly in the ZG from 30-150 days, and not at all in the adrenal medulla. Expression of MRAP α in the ZF supports its physiological role in ACTH activation of the MC2R for glucocorticoid production. Strong MRAP2 expression in the ZG and adrenal medulla indicates that MRAP2 is likely to have physiological roles in the production of adrenal gland-derived mineralocorticoids and catecholamines respectively, and the latter is independent of the MC2R.

1. Webb TR and Clark AJL, Mol Endocrinol 2010; 475

Sources of Research Support: Lottery Health New Zealand; Neurological Foundation New Zealand.

Nothing to Disclose: SR, AH, FL, DG, RNM, KGM

Pub # P1-44

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Development & Epigenetics of Steroid Biology & Neoplasia (1:30 PM-3:30 PM)

Title The Th17 Pathway Influences Susceptibility to Autoimmune Addison Disease

Author String AL Mitchell, K MacArthur, AB Wolff, B Skiningsrud, DE Undlien, ES Husebye, SHS Pearce
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Body Autoimmune Addison's disease (AAD) is a rare, highly heritable endocrinopathy with an estimated [lambda] sibling (ratio of risk to a sibling vs the unrelated background population) of 160-210. The majority of the genetic risk to AAD has yet to be accounted for. Variants in Th17 pathway genes, including IL-21, 22, 23, 17A and 17F and their receptors may be important in the pathogenesis of autoimmune conditions. We used 7 informative tag-SNPs to investigate 4 candidate genes of the Th17 pathway in subjects with AAD: IL17A (rs4711998, rs3819024, rs16882180), IL17RA (rs2241043, rs6518661), IL21 (rs907715) and IL23A (rs11171806). DNA samples from a cohort of 315 UK and 384 Norwegian AAD patients, and 335 UK and 380 Norwegian controls (total 699 AAD patients, 710 healthy controls) were genotyped using a Sequenom iPLEX platform. Ten percent of samples were re-genotyped blind to ensure assay fidelity, all datasets were in Hardy Weinberg equilibrium and McNemar's test confirmed that allele frequencies in the cohorts did not differ significantly. Of the 3 SNPs typed in IL17A, an association with alleles at rs4711998 was seen in the Norwegian AAD cohort (P genotype 0.08, P allele 0.04) and in the cohorts combined (P allele 0.04), however not in the UK cohort. The rs907715 SNP in IL21 was associated with AAD in the UK cohort (P allele 0.01), and in the cohorts combined where the C/C genotype was observed in 319/699 (45.6%) cases compared to 277/710 (39.0%) controls (P genotype 0.01, P allele 0.006), however this association was not replicated in the Norwegian cohort alone (P allele 0.163). The rs11171806 SNP in IL23A was associated with AAD in the UK cohort, where the A/G genotype was found in 31/315 (9.8%) AAD subjects compared to 56/335 (16.7%) controls (P genotype 0.005, P allele 0.007). This SNP was not associated with AAD in the Norwegian cohort (P allele 0.986) and association in the combined cohort failed to reach statistical significance (P allele 0.066). rs22410043 and rs6518661 in IL17RA, and rs3819024, rs16882180 in IL17A were not associated with AAD in any of the cohorts. These data, from the largest collection of AAD patients ever assembled, suggest a contribution to disease susceptibility at the IL17 and IL21 loci, suggesting a role for the Th17 pathway in AAD pathogenesis. We plan to investigate these SNPs in additional AAD patients from other European cohorts to replicate these findings.

Sources of Research Support: Medical Research Council; Euradrenal - EU seventh framework programme.

Nothing to Disclose: ALM, KM, ABW, BS, DEU, ESH, SHSP

Pub # P1-45

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Development & Epigenetics of Steroid Biology & Neoplasia (1:30 PM-3:30 PM)

Title The Effect of APC Mutation on Development of Nonfunctional Adrenocortical Adenoma

Author String T Shiwa, K Oki, T Awaya, S Nakanishi, K Yamane
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Body **(Background)** Familial adenomatous polyposis (FAP) is characterized by the formation of hundreds to thousands of colorectal adenomatous polyps. It is an autosomal dominantly inherited disorder, which results from a germ line mutation in APC (adenomatous polyposis coli) gene. Adrenal adenomas are frequently occurring extraintestinal manifestations of FAP. Although some studies previously showed that the prevalence of adrenal masses in patients with FAP was 7-13%, it was two to four times as high as in the general population with a prevalence of 0.7-4.4%, and many of them could not produce hormones. Therefore, we hypothesized that there would be associations between FAP and nonfunctional adrenocortical adenomas. We assessed the mutation of APC gene in the patients with isolated nonfunctional adrenocortical adenomas. **(Methods)** We prospectively evaluated 90 patients with incidentally detected adrenal masses who were referred to our hospital. The patients who were diagnosed as having cortisol producing adenoma (n=12), aldosterone producing adenoma (n=8), pheochromocytoma (n=5), ganglioneuroma (n=4), cyst (n=1), or asymptomatic metastasis of other tumors (n=4) were excluded. Finally, a total of 54 patients (24 men, 30 women) were identified and diagnosed as having nonfunctional adrenocortical adenomas. In the FAP, one mutated APC allele is transmitted through the germ-line. Subsequent somatic mutations inactivate the second allele and precede the development of many polyps early in the adulthood that evolve systematically into malignant tumors. In such cases, mutation occurs within codon 1286-1513, the so-called mutation cluster region (MCR). We examined codon 1286-1513 including MCR of APC gene by direct sequence analysis. **(Results)** The patient's ages ranged between 33 and 87 years old (mean, 61.9 years). The diameter of adrenal masses varied from 10.0 to 47.9 mm (mean, 20.0 mm). Nine patients had bilateral adrenal adenomas and 5 patients had two or over adrenal adenomas were located on the unilateral adrenal gland. No previously reported and new mutations in codon 1286-1513 of APC gene were detected in all the patients including cases even with multiple adenomas. **(Conclusions)** There was no mutation of APC gene in Japanese patients with nonfunctional adrenocortical adenomas. Thus, the development of isolated adrenocortical adenoma might not be related with APC mutation.

Nothing to Disclose: TS, KO, TA, SN, KY

Pub # P1-46

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Development & Epigenetics of Steroid Biology & Neoplasia (1:30 PM-3:30 PM)

Title Analysis of a *DHCR7* Polymorphism in Type 1 Diabetes, Hashimoto Thyroiditis, Graves Disease, and Addison Disease

Author String Y Moran-Auth, M Penna-Martinez, G Meyer, K Badenhoop, E Ramos-Lopez
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Body 7-dehydrocholesterol (7-DHC) is reduced by a 7-dehydrocholesterol-reductase (7-DHCR) to cholesterol; otherwise 7-DHC is necessary for the isomerization to pre-Vitamin D₃. Many disorders including immune diseases have been associated with Vitamin D insufficiency and recent studies point to associations with the *DHCR7* (rs-12785878) polymorphism. To investigate the role of the *DHCR7* polymorphism we analysed its genotype distribution in patients with type 1 diabetes (T1D), Hashimoto's thyroiditis (HT), Graves[acute] (GD) or Addison[acute]s disease (AD), and healthy controls (HC) and its influence on 25-hydroxyvitamin D₃ (25(OH)D₃). German patients with GD (n=221), HT (n=111) AD (n=330), T1D (n=884) and HC (n=292) were genotyped for the *DHCR7* polymorphism by the Taqman assay. In addition, the 25(OH)D₃ plasma level in patients were measured by radioimmunoassay. Statistical analyses were performed by allele-wise and genotype-wise Chi(x²)-test and plasma levels by Kruskal-Wallis-test.

No significant differences between the allele and genotype frequencies with all studied diseases versus HC were observed. When subjects were stratified into male and females, the allele [ldquo]G[rdquo] (30% vs. 23%, OR=1.38; 95% CI:1.03-1.86) of the rs-12785878 polymorphism was significant more frequent, whereas the allele [ldquo]T[rdquo] (70% vs. 77%, OR=0.72; 95% CI:0.54-0.97) was less frequent in male patients with T1D compared to HC (p=0.03). Furthermore the homozygosity rate [ldquo]GG[rdquo] was higher in male patients with T1D than HC (9% vs. 4%, p=0.05). Significantly lower levels of 25(OH)D₃ were found in young T1D patients carrying the [ldquo]GG[rdquo] genotype than those with the [ldquo]TT[rdquo] genotype (p=0.009).

The allele [ldquo]G[rdquo] of the *DHCR7* polymorphism appeared to be associated with low 25(OH)D₃ level and an increased T1D risk in men. A possible explanation for this sex-specificity in T1D could be an altered steroid precursor synthesis affecting testosterone and associated immune changes.

Sources of Research Support: EU-FP7 grant "EURADRENAL", grant agreement HEALTH F2-2008-201167.

Nothing to Disclose: YM-A, MP-M, GM, KB, ER-L

Pub # P1-47

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title The Role of Androgen Receptor in Postmenopausal Breast Cancer

Author String DR Cochrane, BM Jacobsen, A Jean, A Elias, JK Richer
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Body **Background:** Approximately 30 percent of patients with breast cancers that retain estrogen (ER) and progesterone receptors (PR) do not respond to hormone therapies like tamoxifen or aromatase inhibitors (AI). By profiling breast cancers from neo-adjuvant treatment with either AI alone or AI plus tamoxifen pre- versus post-treatment, we identified increased expression of androgen receptor (AR), genes involved in hypoxia, and a [ldquo]lipogenic profile,[rdquo] as characteristics of non-responsive tumors (Harvell DM et al 2008). **Hypothesis/objective:** We postulate that ER+ breast cancers that fail to respond or become resistant to current endocrine therapies (tamoxifen or AI) may do so because they have switched from growth controlled by estradiol (E2) and ER to growth controlled by liganded AR. We therefore sought to determine if blocking AR activity could serve as a therapeutic intervention for such tumors. While it may be the case that androgens and AR are protective in the pre-menopausal state, in the post-menopausal state, they may contribute to tumor proliferation. **Methods/results:** To test whether androgens are proliferative, we used breast cancer cells that express ER and AR such as MCF7 cells and a cell line that we recently isolated that contains more AR than ER. Our data indicate that although DHT does slightly inhibit E2-mediated proliferation, DHT alone is proliferative in cells such as MCF7 with both ER and AR, and is even more proliferative than E2 when AR is more abundant than ER. We found that while both the anti-androgen bicalutamide and the triple acting, non-steroidal, AR antagonist MDV3100 block DHT and R1881-mediated proliferation of breast cancer cells, we made the novel observation that MDV3100, but not bicalutamide, inhibits E2-mediated proliferation of breast cancer cells. Thus, certain AR antagonists may have potential therapeutic utility in breast cancers that are ER and/or AR positive. We are performing additional studies on the ability of the new generation AR antagonist to block estrogen and/or androgen mediated proliferation *in vivo*. **Conclusion:** Liganded AR may drive proliferation of some breast cancers in post-menopausal women, particularly those that do not respond initially to AI or fail on AI. Anti-androgens, such as MDV3100, may be particularly useful to treat patients whose tumors fail to respond to traditional endocrine therapy despite being ER+, or who have ER-/AR+ tumors.

Nothing to Disclose: DRC, BMJ, AJ, AE, JKR

Pub # P1-48

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Targeting Androgen Receptor in Estrogen Receptor-Negative Breast Cancer

Author String M Ni, Y Chen, E Lim, ST Bailey, Y Imai, XS Liu, M Brown
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Body Sex steroid hormones are critical for the development and progression of cancers of reproductive organs including the uterus and breast in women and the prostate in men. Steroid hormone receptors are crucial components of steroid hormone signaling that act as transcription factors to regulate gene expression, however their function contributes to hormonal carcinogenesis. Achievements in biological studies of estrogens and estrogen receptor (ER) led to remarkable progress in breast cancer therapy, such as anti-estrogen regimens and the clinical application of ER antagonists, e.g. tamoxifen and fulvestrant. While patients with ER-positive breast tumors obtain benefit from these advancements, there is still no effective targeted therapy for most ER-negative breast cancer patients. The ER-negative breast cancers account for 25-30% of all breast cancer cases and generally have a poor prognosis and little benefit from current endocrine therapy. Recent studies have found that androgen receptor (AR) is expressed in 60-70% of human breast tumors independent of the ER status. Importantly, while androgens inhibit the growth of ER+/AR+ breast cancer cells, they stimulate the growth of those that are ER-/AR+. However, the mechanism by which androgens and AR function to regulate breast tumor growth remains largely unknown. Analysis of gene expression microarray datasets from human breast tumors reveals enriched expression of AR in the ER-negative breast tumors that over-express HER2. Through genome-wide analysis of the AR binding sites and androgen-regulated gene expression profiles in ER-/HER2+ breast cancer cells, we identified that AR mediates activation of oncogenic Wnt and HER2 signaling pathways through direct transcriptional up-regulation of WNT7B and HER3 in an androgen-dependent manner. Importantly, we demonstrate that FOXA1 and beta-catenin act as AR coactivators in inducing HER3, leading to the enforced HER2/HER3 signaling. Pharmacological inhibition of AR, Wnt or HER2 pathways impairs HER3 activation and androgen-stimulated tumor cell growth. Collectively, these findings not only highlight a previously unappreciated role of androgens and AR in breast cancer, but also reveals the mechanistic basis for targeting AR as a novel therapeutic opportunity for patients with invasive ER-/HER2+/AR+ breast tumors.

Sources of Research Support: Department of Defense Award W81XWH-10-1-0037 to MN.

Nothing to Disclose: MN, YC, EL, STB, YI, XSL, MB

Pub # P1-49

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title First Evidence of the Up-Regulatory Effect of Androgens on DAX-1 Gene Expression: Is This a Novel Mechanism for Inhibiting Breast Cancer Cell Proliferation?

Author String M Lanzino, C Morelli, D Sisci, I Casaburi, P Maris, C Capparelli, P Avena, S Ando
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University of Calabria, Arcavacata di Rende, Italy

Body Breast cancer development and progression depend on a complex cross-talk between several hormone transduction pathways. Estrogens are known to stimulate breast cancer cell growth and survival, and estrogen receptor (ER) expression in mammary tumors is widely considered as a favourable prognostic marker for both disease outcome and anti-estrogen treatment responsiveness. Despite the growing body of evidence indicating a protective role of androgen receptor (AR) in breast tumorigenesis, the mechanism underlying the inhibition of the *in vivo* and/or *in vitro* breast cancer cell growth by activated AR, remains poorly defined. Our previous studies showed that, in AR and ER positive breast cancer cells MCF-7, the AR ligand DHT inhibits serum as well as estrogen-dependent cell proliferation directly by inhibiting the cyclin D1 gene expression, and indirectly, by decreasing ER transcriptional activity in presence of over-expressed AR. The orphan nuclear receptor DAX-1 is widely distributed in the reproductive and endocrine systems where it acts as a transcriptional corepressor of steroidogenic factor 1 (SF-1) in the induction of steroidogenic genes, such as aromatase. However, DAX-1 functions extend beyond regulation of SF-1-dependent genes as it also inhibits ligand-dependent transactivation by agonist-bound nuclear receptors like AR and ER. DAX-1 also plays a critical role in the AR-mediate repression of cyclin D1 expression in breast cancer cells. Nevertheless, there are few reports on how DAX-1 expression is regulated. Here we report first evidences that, in MCF-7 cells, DHT-activated AR induces endogenous DAX-1 expression, as indicated by the increase of DAX-1 mRNA and protein levels, and the enhancement of *DAX-1*-promoter activity. The results of DAPA, EMSA and ChIP analyses strongly suggest that this up-regulatory effect is ligand-dependent and it is mediated by binding of AR to a putative Androgen Response Element (DAX-1-ARE), located in the DAX-1 promoter. Identification of this DAX-1-ARE could allow to define DAX-1 as an androgen target gene in breast, thus providing a new insight into understanding the basic mechanism of DAX-1 and AR interplay. Since in mammary mouse epithelial cells, DAX-1 expression has been shown to influence cell growth by reversing ER proliferative effects, our study is also expected to provide clues for a better comprehension of the AR-dependent inhibition of breast cancer cell proliferation.

Nothing to Disclose: ML, CM, DS, IC, PM, CC, PA, SA

Pub # P1-50

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Early B-Cell Factor 1 (EBF1) Regulated Estrogen Receptor beta Activity on a Genomic Scale

Author String T Le, G Greene
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Body Considerable effort by numerous laboratories has resulted in an improved understanding of estrogen and SERM action mediated by the two estrogen receptors, ER α and ER β . However, many of the targets for ER β in cell physiology remain elusive. Here we describe a study that thoroughly investigates the transcriptional targets and functions of ER β . MCF7 C4-12 cells, which do not express either ER, were engineered to stably express a FLAG-tagged ER β . In this cell model, ER β transcriptional activity can be studied in an ER α -independent background. According to our ChIP-seq results (chromatin immunoprecipitation followed by deep sequencing with Solexa technology), <15% ER β binding sites were within proximal promoter regions of putative target genes. The majority of binding events occurred either in intronic (45%) or intergenic (36%) regions. While the most prevalent binding motif was the canonical ERE, ~30% of ER β binding regions also carried the binding motif of EBFs (Early B-cell Factor 1). This result suggests that EBF1 might potentiate or attenuate ER transcriptional activities on a global scale. V5-tagged EBF1 was co-immunoprecipitated with FLAG-tagged ER β , demonstrating a direct interaction in a ligand independent manner. Moreover, in our luciferase reporter assay, EBF1 down-regulated both ER α and ER β activities. Interestingly, ER constructs carrying the AF1 domain of ER β (full length ER β and ER β / α chimera) were more affected compared to those carrying ER α AF1 domain (full length ER α and ER α / β chimera). These results, at least to our knowledge, are the first to indicate crosstalk between EBF and ER transcriptional activities on a large scale. Because EBF2, EBF3, and EBF4 transcripts were found to be abundant in MCF7 C4-12 cells expressing FLAG-tagged ER β (EBF3 > EBF2/EBF4 > EBF1), the roles of these proteins in influencing ER β activity are under active investigation. Moreover, in conjunction with gene expression profiling, still to be completed, our results should reveal a global picture of ER β transcriptional activity, as well as the role of EBF proteins in this process.

Nothing to Disclose: TL, GG

Pub # P1-51

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Breast Cancer Initiation, Tumor Induction and Growth Are Promoted by 5 α -Dihydroprogesterone (5 α P) and Inhibited by 3 α -Dihydroprogesterone (3 α HP) in the Microenvironment

Author String JP Wiebe
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Body Our previous studies showed that human breast tissues convert progesterone (P) to 5 α -dihydroprogesterone (5 α P) and 3 α -dihydroprogesterone (3 α HP) and that tumorous tissues produce more 5 α P and less 3 α HP than normal tissues [1], due to differences in expression of P metabolizing enzymes [2]. Numerous in vitro studies on various breast cell lines have shown that 5 α P stimulates, whereas 3 α HP suppresses, proliferation and detachment of human breast cells, due to opposing actions on mitosis, apoptosis, actin polymerization, adhesion plaque formation, and Bcl-2, Bax and p21 expression [3,4]. The opposing actions of 5 α P and 3 α HP were observed on all human breast cell lines studied, regardless of estrogen and P receptor levels, and whether cells are tumorigenic or 'normal'. The objective of the current studies was to determine if 5 α P and 3 α HP affect human breast cell tumor induction and growth in xenografts and to measure the levels of these hormones in matched serum and tumor tissues. Breast cancer (MDA-MB-231) cells, were surgically implanted into the mammary fat pads of 6 week old SCID mice which received two sc injections of vehicle without (control), or with either 5 α P, 3 α HP, or 5 α P+3 α HP. Four experiments were conducted with essentially similar results. In comparison with the controls, tumors developed significantly earlier, in more mice and more rapidly (about 19-fold in volume) in the 5 α P group; tumors developed significantly later, in fewer mice and more slowly (0.54-fold in volume) in the 3 α HP group, and the stimulatory effects of 5 α P were significantly abrogated by 3 α HP in the 5 α P+3 α HP group. Measurements in 8 mice of 5 α P and 3 α HP (by RIA) showed that in tumors, 5 α P levels were more than 3-fold greater, and 3 α HP levels were less than half those in serum, resulting in 5 α P:3 α HP ratios of 1.3 in serum and 10.6 in tumors. Measurements of 5 α P and 3 α HP in tumors by GC-mass spectrometry indicated that 5 α P levels were 23-33-fold higher than 3 α HP levels, confirming the RIA findings that 5 α P levels greatly exceed the 3 α HP levels in tumors. Concentrations of P did not vary significantly between serum and tumor. The findings provide the first in vivo evidence that the P metabolites, 5 α P and 3 α HP, act as potent endogenous mammary cancer regulating hormones and that the elevated 5 α P:3 α HP ratios in the microenvironment of breast tumors may be responsible for neoplasia and tumorigenesis.

[1] Wiebe JP et al., Cancer Res 2000; 60:936

[2] Lewis MJ et al., BMC Cancer 2004; 4:27

[3] Wiebe JP, Endocr Rel Cancer 2006; 13:717

[4] Wiebe JP et al., J Steroid Biochem Mol Biol 2010; 118:125

Sources of Research Support: Canadian Institutes for Health Research; Canadian Breast Cancer Research Alliance; Susan G Komen for the Cure.

Nothing to Disclose: JPW

Pub # P1-52

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Role of Polycomb Repressive Complexes in Tamoxifen-Resistant Breast Cancer

Author String K Xu, Z Wu, XS Liu, MA Brown
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Body Tamoxifen has been the golden standard of endocrine therapy in ER-positive breast cancer since 1970's. About 70% of breast tumors express ER, and among them 70-80% respond positively to tamoxifen treatment. However, most of the patients develop de novo or acquired resistance after 1-3 years of hormonal manipulations, and the tumors start to grow despite continuing tamoxifen treatment. Several studies have indicated the important role of epigenetic changes in the development of tamoxifen resistant breast tumors, and the polycomb group protein EZH2 is one of the signatures that are up-regulated in tamoxifen resistant breast tumors. EZH2 is a histone methyl transferase towards K27 of histone H3, which is responsible for transcriptional silencing of target genes. It is the catalytic subunit of Polycomb Repressive Complexes, and it has to reside within the complexes with SUZ12 and EED to exert the maximum activity. It is known that EZH2 is overexpressed in invasive and metastatic breast tumors compared to the normal samples, and the higher amount of EZH2 is strongly associated with poorer clinical outcomes after treatments.

To explore the potential role of Polycomb Repressive Complexes in breast cancer, we knocked down EZH2 in MCF7 cells, and found that combining EZH2 silencing with tamoxifen treatment significantly inhibited breast cancer cell growth comparing to EZH2 knocking down or drug treatment alone. We then screened the direct targets of EZH2 by gene expression microarray and genome-wide ChIP-sequencing, and discovered one of its direct targets is PAX2, which has been indicated to play an important role in regulation of ERBB2 expression and tamoxifen resistant development. We further confirmed the down-regulation of PAX2 by the core subunits of Polycomb Repressive Complexes, and the occupation of these components as well as the repressive histone mark H3K27 trimethylation at the cis-regulatory elements we identified from our ChIP-sequencing results. We also consistently found the positive correlation between ERBB2 and EZH2 levels either in our cell models or from publicly available human breast cancer datasets. Taken together, our preliminary data has indicated an important role of Polycomb Repressive Complexes in tamoxifen resistant breast cancer, and the epigenetic regulation of important genes such as PAX2 in the development. Our data suggests the potential application of epigenetic drugs in tamoxifen resistant breast tumors.

Nothing to Disclose: KX, ZW, XSL, MAB

Pub # P1-53
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)
Title Interplay between Skp2, p38MAPK and Oct-3/4 Regulates Tamoxifen Resistance in Breast Cancer Cells
Author String S Bhatt, JD Stender, BS Katzenellenbogen
University of Illinois at Urbana-Champaign, Urbana, IL; University of Illinois at Urbana-Champaign, Urbana IL

Body Tamoxifen has shown great success in the treatment of estrogen receptor-positive breast cancers. However, long term treatment often leads to acquired resistance where cells become growth-stimulated rather than suppressed by tamoxifen. While estradiol (E2) and trans-hydroxy-tamoxifen (TOT) treatment of breast cancer cells results in recruitment of estrogen receptor (ER α) to common and distinct genomic sites, the mechanisms and proteins involved in tamoxifen preferential recruitment of ER α remain poorly defined. Therefore, to identify factors that recruit ER α to genomic binding sites specifically in response to TOT as compared to E2, we searched for enriched motifs in genomic binding sites bound by ER α preferentially in the presence of TOT vs. E2. By computational motif analysis, we found the motifs for Nkx3-1 and Oct-family to be enriched in TOT-preferential binding sites and confirmed by chromatin immunoprecipitation (ChIP) that Oct-3/4 directed recruitment of ER α to a subset of TOT preferential genomic binding sites. Oct-3/4 expression was basally repressed by Nkx3-1, and we observed that TOT treatment of MCF-7 breast cancer cells resulted in Nkx3-1 degradation through p38MAPK-dependent phosphorylation of the E3 ligase, Skp2 at its Serine-64 residue, as observed by mass-spectrometry and in-cell kinase assays. Phosphorylated Skp2 targeted Nkx3-1 for proteasomal degradation, resulting in activation of Oct-3/4 gene expression. Oct-3/4 also participated in ER α gene regulation along with p38MAPK and Skp2 in a tamoxifen-dependent manner leading to gene activation of candidate mRNAs such as NFATC4. Consistent with this, Oct-3/4 levels, which were highly elevated in TOT-resistant breast cancer cells, appeared critical for the regulation of TOT sensitivity and tamoxifen driven growth of these cells. Collectively, our findings establish a critical role for Oct-3/4 specified recruitment of ER α to genomic binding sites, driving gene activation exclusively in response to tamoxifen, as a novel underlying mechanism for driving tamoxifen resistance in breast cancer cells. We therefore, propose this regulatory pathway as potential target for therapeutic intervention to render tamoxifen resistant breast cancers amenable to endocrine therapy.

Sources of Research Support: NIH and The Breast Cancer Research Foundation.

Nothing to Disclose: SB, JDS, BSK

Pub # P1-54

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Regulation of Nuclear Respiratory Factor 1 by Estradiol and Tamoxifen in Endocrine-Resistant Breast Cancer Cells

Author String KH Luken, MM Ivanova, CM Klinge
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Body Nuclear Respiratory Factor-1 (NRF-1) is a transcription factor that increases expression of genes involved in mitochondrial biogenesis and function, as well as increasing the transcription of genes involved in other cellular functions. Previous work in our lab has shown that estradiol (E2) stimulates the transcription of NRF-1 and increases mitochondrial biogenesis in MCF-7 (ER+, Tamoxifen (TAM)-sensitive) breast cancer cells. Surprisingly, we have also shown that 4-hydroxyTAM (4-OHT), the active metabolite of tamoxifen, also increases NRF-1 expression in MCF-7 and T47D breast cancer cells. However, an increase in NRF-1 target genes was not detected in 4-OHT treated cells, as NRF-1 is not transcriptionally active due to a lack of phosphorylation because 4-OHT inhibits AKT activity. The current model is that in TAM-sensitive breast cancer cells E2-occupied ER α interacts with the promoter of NRF-1 to increase the transcription of NRF-1 while 4-OHT-occupied ER β activation of NRF-1 transcription requires not only the ERE but the adjacent AP-1 site. We have new data indicating that E2 and 4-OHT increase NRF-1 expression in ER-independent, TAM-resistant cell lines (MCF7-LCC1, MCF7-LCC2, MCF7-LCC9, and MCF7-LY2). NRF-1 expression data in response to E2 or 4-OHT treatment in the TAM-resistant cell lines will be correlated with the expression of NRF-1 target genes. Additionally, basal oxygen consumption will be correlated with basal NRF-1 expression. Overall, these data show that NRF-1 expression is increased by E2 and 4-OHT in cells that are E2-independent and TAM-resistant, giving the cells the ability to increase mitochondrial activity, and thus a growth advantage.

Sources of Research Support: NIH R01 DK53220 to CMK. KHL is supported by DOD CMDRP BC100782.

Nothing to Disclose: KHL, MMI, CMK

Pub # P1-55

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Exposure to Tamoxifen Enhances Apoptosis-Induced Killing of Human Breast Cancer Cells by Monocytic Immune Cells

Author String M Ramzy, SM Aronica
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Body Exposure of breast cancer cells to a number of agents, including chemokines and hormones has been shown to influence their growth, migration, and adherence. We have shown previously that select chemokines can disrupt breast cancer cell viability, migration and adherence through regulation of the expression of various molecules, including integrins. We set out to determine whether these compounds could influence the ability of breast cancer cells to serve as targets for destruction by immune cells. MCF-7 or 231 breast cancer cells were treated with control vehicle alone, with tamoxifen or progesterone alone, or with one of the following chemokines: IP-10 (CXCL10), SDF1 (CXCL12) or MSP (macrophage stimulating protein) for 48 hours. Half way through the incubation period, murine monocytic cells or murine T-lymphocytes were added to the treated breast cancer cells in culture. Following the full treatment duration, combined cell populations were harvested from each treatment flask, and divided into two groups for analysis. Samples were stained with labeled anti-HER2/neu antibodies, or with apoptosis detection reagents. Expression of HER2, indicative of human cancer cells in the sample, was determined using a Guava PC flow cytometer and Express software. Apoptosis levels were determined using Nexin software and a commercially available assay kit. Both MCF-7 and 231 cells expressed HER2 on their surface. This was not shown for murine monocytes and T cells. Exposure of MCF-7 cells to the chemokines did not influence cell behavior, but apoptosis levels significantly increased for MCF-7 cells treated with tamoxifen for 24 hours and then exposed to monocytic cells. The rise in apoptosis correlated with reductions in HER2/neu positive cells within the combined cell population, indicating loss of human cells. This enhanced apoptosis of MCF-7 breast cancer cells was not shown for cells exposed to murine T cells. Neither exposure to the various reagents nor immune cells used in our studies served to alter apoptosis levels for 231 cells. Our results suggest that the effects of tamoxifen on estrogen-positive breast cancer might include mechanisms designed to evoke killing activity of monocytic cells. Ongoing studies address mechanisms through which tamoxifen may alter the apoptosis-inducing activity of monocytic cells, to determine if cell adherence is associated with this activity, and an assessment of whether this also occurs in human monocytes exposed to tamoxifen.

Nothing to Disclose: MR, SMA

Pub # P1-56

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title A MicroRNA and TP63 Circuit Regulated by Estrogen Receptor-alpha and ERK2 That Controls Breast Cancer Proliferation and Invasiveness Properties

Author String Z Madak-Erdogan, K Kim, BS Katzenellenbogen
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Body miRNAs play crucial roles in cancer biology and have regulatory functions in proliferation, differentiation and survival of tumor cells. We have investigated the regulation of miRNAs by estradiol (E2) and growth factors through estrogen receptor-alpha (ER α) and extracellular signal-regulated kinase 2 (ERK2), and their physiological impact on target gene regulation and the phenotypic properties of breast cancer cells. We identified nine miRNA-encoding genes harboring overlapping ER α and ERK2 binding sites in a 50 Kb window around their transcription start sites in MCF-7 cells. ER α and ERK2 were shown to directly bind to the overlapping binding sites near the E2-upregulated miRNAs and to be required for transcriptional induction of these miRNAs as well as for their E2-mediated regulation. We also identified tumor protein p63 (TP63), a target gene of a subset of E2-regulated miRs, and showed that TP63 plays an important role in E2-mediated cellular responses by increasing breast cancer cell proliferation and *in vitro* invasion controlled by the action of one of these miRs, miR-196a2. The expression of Δ Np63a was correlated inversely with ER α levels in several breast cancer cell lines, and we found that invasion and tumor growth properties were reversed by enforced miR-196a2 expression in ER α negative MDA-MB-231 breast cancer cells. These results imply that the regulation of miR-196a2 by ER α and/or ERK2 signaling in breast cancer is associated with different molecular subtypes of breast cancer, possibly mediated through differential TP63 expression affecting tumor cell growth and invasion ability. The findings suggest a potential tumor-suppressive treatment strategy to alleviate the aggressive behavior and poor prognosis of the ER α -negative basal-like breast cancer subtype by manipulating the miR-196a2 and TP63 circuit in these breast cancer cells.

Sources of Research Support: Grants from The Breast Cancer Research Foundation (BSK) and the NIH (P50 AT006268, BSK, and T32ES007326, ZME).

Nothing to Disclose: ZM-E, KK, BSK

Pub # P1-57

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Role of the Cofactor RIP140 in the Anti-Inflammatory Actions of the Progesterone Receptor in Breast Cancer

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Body Progesterone has been shown to have anti-inflammatory properties in the uterus and more recently in cellular models of breast cancer. Our lab has characterized a set of 22 inflammatory genes that are induced by Interleukin-1 β (IL-1 β) and repressed by co-treatment with the progestin R5020 in T47D breast cancer cells (1). The induction of these genes occurred through Nuclear Factor-[kappa]B (NF-[kappa]B), a transcription factor thought to be involved in the etiology of breast cancer. Upon further investigation, we found that progestin mediated repression of a subset of these genes, including the chemokine (C-C motif) ligands (CCL) 4 and 20 was reversed by an inhibiting peptide targeting the AF-2 domain of the progesterone receptor (PR), a region which preferentially binds to LXXLL motifs. Recently, the cofactor RIP140, which contains 9 LXXLL motifs, has been shown to be a coactivator of the NF-[kappa]B subunit RelA and to be necessary for the induction of inflammatory cytokines/chemokines (2). We were interested in whether the anti-inflammatory actions of progestins are mediated through disruption of the RIP140-RelA interaction. A number of genes including, Interleukin-8 (IL-8) as well as CCL2, 4, and 20 were repressed by RIP140 siRNA. Upon IL-1 β treatment, RIP140 and the NF-[kappa]B cofactor CBP were recruited to a consensus NF-[kappa]B site at the CCL20 promotor by chromatin immunoprecipitation. However, R5020 co-treatment inhibited the recruitment of both RIP140 and CBP to the promotor. This suggests that progestins inhibit the recruitment of NF-[kappa]B cofactors to RelA possibly through interfering with the recruitment of RIP140. We have also shown that dexamethasone represses the same inflammatory cytokines/chemokines in T47D, suggesting that both GR and PR may inhibit these genes via similar mechanisms.

(1) Kobayashi, et al., Mol Endo 2010; 24:2292-2302
(2) Zschiedrich, et al., Blood 2008; 112:264-276

Sources of Research Support: Department of Defense Breast Cancer Fellowship W81XWH.

Nothing to Disclose: JPS, SK, DPM

Pub # P1-58

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Effects of Fibroblast Growth Factor-8 on Breast Cancer Cell Mitosis by Regulating Bone Morphogenetic Protein and Estrogen Receptor Actions

Author String F Otsuka, H Masuda, T Miyoshi, N Tsukamoto, M Takano, K Inagaki, Y Matsumoto, H Makino, H Doihara Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Body Recent studies have demonstrated the presence of TGF β signaling in mammary cells. There have been several studies showing the expression of BMPs in breast cancer cells, and their possible roles in breast cancer development and in bone metastasis have been suggested. We reported that BMPs have inhibitory effects on estrogen-induced mitosis of breast cancer cells by inhibiting MAPK and estrogenic enzyme expression (JOE 2008). These suggest the existence of a functional crosstalk between the BMP system and estrogen receptor (ER) actions in breast cancer cells. FGF-8 is found to be expressed in human breast, prostate and ovarian tumors. In breast and prostate cancer cells, FGF-8 has been shown to induce morphological change and increase cell proliferation and tumor growth. On the basis of results showing that FGFs and BMPs regulate cell differentiation cooperatively in a cell/tissue-dependent manner, the effects of FGF-8 on breast cancer cell proliferation caused by estrogen were studied using human breast cancer MCF-7 cells. MCF-7 cells express ER α , ER β , FGF receptors, and BMPR-Smad signaling molecules. Estradiol stimulated MCF-7 cell proliferation in a concentration-responsive manner, whereas BSA-bound estradiol had a weak effect on MCF-7 cell mitosis. It is notable that estrogen-induced proliferation was enhanced in the presence of FGF-8. It was also revealed that FGF-8 increased the expression levels of ER α , ER β and aromatase mRNAs, while estradiol reduced the expression levels of ERs, aromatase and steroid sulfatase in MCF-7 cells. FGF-8-induced phosphorylation of FGFR was augmented by estradiol. FGF-8-induced activation of MAPKs and AKT signaling was also upregulated by estrogen. Interestingly, FGF-8 suppressed BMP-7 actions that are linked to mitotic inhibition by activating the cell cycle regulator cdc2. FGF-8 was revealed to inhibit BMP-Smad1/5/8 activation by suppressing expression of BMP type-II receptors and by increasing expression of inhibitory Smads. Collectively, FGF-8 acts to facilitate cell proliferation by enhancing endogenous estrogenic actions as well as by suppressing BMP receptor signaling in ER-positive breast cancer cells. Given the findings that FGF-8 and FGF receptor expression is increased in human breast cancer tissues, suppression of endogenous FGF-8 functions and/or enhancement of the functional BMP system could be a possible therapeutic strategy to inhibit the development of estrogen-responsive breast cancer.

Nothing to Disclose: FO, HM, TM, NT, MT, KI, YM, HM, HD

Pub # P1-59

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title The Silencing Mediator of Retinoic Acid and Thyroid Hormone Receptor (SMRT) Coregulator Promotes Breast Carcinogenesis through Multiple Cellular Pathways

Author String JK Blackmore, V Chaubal, S Karmakar, CL Smith
Baylor College of Medicine, Houston, TX

Body Aberrant regulation of nuclear receptor coregulators is associated with a wide variety of human diseases. For instance, steroid receptor coactivator-3 (SRC-3) is an oncogene and increased SRC-3 expression is found for a significant number of human breast tumors. We recently determined that the silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) coregulator could interact with SRC-3, increasing its intrinsic transcriptional activity and association with an estrogen receptor- α (ER α) target gene, cyclin D1 (1). Expression of SRC-3 and SMRT were also positively correlated in a small cohort of breast tumors. We therefore wanted to examine the impact of SMRT in breast cancer using control versus SMRT-specific siRNA in ER α -positive (MCF-7) and ER α -negative (MDA-MB-231) breast cancer cell lines. Depletion of SMRT reduced proliferation of MCF-7 cells grown as a monolayer on plastic under basal, estradiol (E2) or 4-hydroxytamoxifen (4HT) treatment conditions. The relative agonist activity of 4HT with respect to cell growth was not increased by SMRT depletion. In contrast, growth of MDA-MB-231 cells was not reduced by SMRT depletion. Flow cytometric analysis of cell cycle distribution revealed the E2-induced shift in synchronized populations from the G1 to S/G2/M phase was compromised in SMRT-depleted MCF-7 cells, consistent with a loss of estrogen induction of cyclin D1 expression in these cells. Moreover, a hormone independent increase in the percentage of SMRT-depleted MCF-7 cells in the subG₀ population was detected suggesting an increase in apoptosis in SMRT depleted cells that was confirmed by Cell Death ELISA, annexin V staining, and PARP cleavage. Short hairpin RNA technology was used to generate MCF-7 cells with stable knockdown SMRT which produced fewer colonies in soft agar assays in comparison to control cells indicating that SMRT promotes anchorage-independent growth. The number of mammospheres formed by SMRT-depleted MCF-7 cells also was reduced compared with control cells and their structures were less well organized. Collectively, these data reveal that SMRT impacts breast tumorigenesis through multiple pathways that inhibit apoptosis, promote cell proliferation and promote anchorage-independent cell growth, and suggest SMRT is a potential therapeutic target for breast cancer.

(1) Karmakar, S et al., Mol Endo 2010; 24:1187

Sources of Research Support: NIH Grant DK53002 awarded to CLS.

Nothing to Disclose: JKB, VC, SK, CLS

Pub # P1-60

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Activation of the Human Gonadotropin-Releasing Hormone Receptor (GnRHR) Promotes Actin Polymerization and Adhesion in MDA-MB-231 Cells Via the Small G Protein Regulator p190RhoGAP

Author String G Maya-Nunez, M Huerta-Reyes, A Ulloa-Aguirre, J Tortoriello, A Aguilar-Rojas
Instituto Mexicano del Seguro Social, Xochitepec, Mexico; Instituto Mexicano del Seguro Social, Mexico City, Mexico

Body In the present study, we analyzed the effects of GnRHR activation on actin polymerization (F-actin) and adhesion in MDA-MB-231 (MDA) breast cancer cells transiently expressing the wild-type (WT) GnRHR and the GnRHR/[Delta]k191 mutant (which is expressed at levels higher than the WT receptor). Radioligand binding assays and GnRH analog (Buserelin)-stimulated measurements of inositol phosphate production corroborated the presence and functionality of the expressed receptors. Compared to control cells, GnRH analog-stimulated MDA cells showed a significant increase in the amount of F-actin; higher levels of F-actin were found in cells expressing the GnRHR/[Delta]k191. Although confocal microscopy of cells expressing both GnRHRs did not show any change in morphology after Buserelin stimulation, high amounts of stress fibers were visualized. Since previous studies have shown that the GnRHR is able to activate the GTPase RhoA (a protein responsible of stress fiber formation), WT GnRHR and the Rho-activating protein p190RhoGAP (p190) were coexpressed. Cells overexpressing both proteins exhibited an additive effect on F-actin levels while cells cotransfected with the WT GnRHR and a dominant negative form of p190RhoGAP ([Delta]p190) showed lower levels of the actin polymer. To determine whether the high amounts of stress fibers observed have an effect on adhesion capacity, adhesion assays were performed. These experiments showed that activation of the WT and [Delta]k191 GnRHRs promoted a substantial increase in this particular end point; similarly, cells cotransfected with the WT GnRHR plus p190 also showed an additive effect. In cells cotransfected with the WT GnRHR plus [Delta]p190, the adherence ability in the presence of Buserelin was substantially decreased. Finally, the migration capacity of MDA cells was evaluated in Buserelin-stimulated cells. Wild-type and mutant GnRHR-expressing cells exhibited a low capacity to respond to chemoattraction, whereas in those cotransfected with the WT GnRHR plus p190 a major decline in migratory capacity was observed probably due to the high adhesion of cells to the substrate. Cells cotransfected with the WT GnRHR plus [Delta]p190 were completely unable to migrate. These data reinforce the idea that the effect of GnRH on the cytoskeleton may be exerted through activation of the protein RhoA via p190. These molecules promote actin polymerization and increase adhesion to the substrate, which in turn decrease the migratory ability of MDA-MB-231 cells.

Sources of Research Support: Grants 83142 from CONACyT, México, and grant 2007-3606-14 from the FIS-Instituto Mexicano del Seguro Social, México (to A.A-R.).

Nothing to Disclose: GM-N, MH-R, AU-A, JT, AA-R

Pub # P1-61

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Small Molecule Inhibitors of Estrogen Receptor Action Identified by High-Throughput Screening

Author String ND Andruska, MM Cherian, C Mao, MT Cherian, DJ Shapiro
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Body To identify novel pathways and interactions important for estrogen receptor α (ER α) action, we carried out an unbiased cell-based screen of 150,000 small molecules for inhibitors of ER-mediated transactivation and growth of breast cancer cells. Surprisingly, most of the 23 small molecules that specifically inhibited estrogen dependent growth of MCF-7 cells with IC₅₀s <1 [micro]M targeted a pathway not previously closely linked to ER action. In contrast, only 3 of the 23 small molecules had properties consistent with possible action as inhibitors of coactivator binding-the primary target site in most studies designed to identify small molecule inhibitors of ER. We screened for small molecules that inhibited ER-mediated expression of a stably transfected ERE-luciferase reporter gene in ER positive T47D, human breast cancer cells. This unbiased cell-based screen asks the cell to tell us what interactions and pathways are susceptible to targeting by small molecules-with a readout of inhibition of 17 β -estradiol-ER mediated gene expression. Candidate inhibitors were evaluated in more detail for the ability to inhibit estrogen-dependent growth of ER positive MCF-7, human breast cancer cells, with little or no effect on the growth of ER-negative MDA-MB-231 cells, or on transactivation by androgen receptor. The ~40 most potent inhibitors were further tested for specificity using non-tumorigenic, ER negative MCF-10A human mammary epithelial cells. The screening conditions were designed to minimize the possibility that our small molecule hits would be ligands that compete with estradiol for binding in the ligand-binding pocket of ER α . Since competitors would be expected to show reduced inhibition of ER action at high concentrations of 17 β -estradiol, we tested ~40 of the most potent hits for the ability to inhibit ER mediated transactivation in the presence of 1 nM estradiol and 500 nM estradiol. None of the inhibitors appear to be competitor ligands. 23 compounds, comprising 18 distinct structural families, specifically inhibited the estrogen dependent growth of MCF-7 cells with IC₅₀s <1 [micro]M. Inhibitors with different modes of action exhibited a broad range of potency, with highly potent inhibitors targeting several different sites. These studies indicate that an unbiased approach using a cell-based readout has the potential to identify novel pathways and interactions that influence steroid receptor activity.

Sources of Research Support: NIH DK071909.

Nothing to Disclose: NDA, MMC, CM, MTC, DJS

Pub # P1-62

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Proinsulin Exerts Biological Effects in Human Cancer Cells through Isoform A of the Insulin Receptor

Author String R Malaguarnera, A Sacco, G Pandini, R Vigneri, A Belfiore
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Body Insulin resistance, a major risk factor for cancer development and poor prognosis, is characterized not only by hyperinsulinemia but also by increased proinsulin (Pro-I):insulin ratio. Available evidences indicate that insulin may stimulate proliferation in cancer cells mainly through the overexpressed isoform A of the insulin receptor (IR-A), while Pro-I is generally considered an inactive pro-hormone because of its low metabolic activity. Whether Pro-I may differentially signal and elicit biological effects through the two IR isoforms (IR-A vs. IR-B) is unknown.

We evaluated the signaling and the biological effects mediated by Pro-I in human cancer cells, including SKUT-1, MDA-MB-157, PC-3, and HepG2, characterized by different IR-A content and relative abundance. We also studied R- fibroblasts lacking IGF-IR and transfected with IR-A or IR-B.

In all cancer cell lines Pro-I stimulated cell proliferation, migration and cell cycle progression dose-dependently, starting at 1nM. At 10nM Pro-I was almost as effective than insulin. IR autophosphorylation in response to Pro-I was lower than after insulin and correlated with IR-A abundance. IGF-IR was not activated. ERK1/2 and p70S6K were both activated by Pro-I and insulin to a similar extent, while Akt activation was noticeably lower after Pro-I than after insulin. We then assessed the in vitro ability of immunopurified IR-A, IR-B and IGF-IR to be activated by Pro-I. We found that only IR-A was activated by Pro-I with relatively high affinity, while both IR-B and IGF-IR were not.

Pro-I should be regarded, therefore, as a selective IR-A ligand that may exert various biological effects in cancer cells. Increased circulating Pro-I in insulin resistant patients may cause unbalanced stimulation through IR-A.

Nothing to Disclose: RM, AS, GP, RV, AB

Pub # P1-63

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title 2-Methoxyestradiol Modulation of Estrogen Receptor and Tumor Suppressor p53 in Breast Cancer Cells

Author String AE Siebert, AL Sanchez, S Dinda, VK Moudgil
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Body An endogenous estradiol (E₂) metabolite, 2-methoxyestradiol (2-ME₂), has been shown to exhibit antiangiogenic and antitumor effects. This compound has potential as a cytostatic treatment of tumor growth. We have studied the tumor suppressor protein p53 and estrogen receptor (ER) as possible molecular targets of 2-ME₂. Despite being a natural metabolite of E₂, the antiproliferative effects of 2-ME₂ have been reported to be independent of ER status and not ER-mediated. We have examined potential alterations in p53 and ER α in response to 2-ME₂ in T47D breast cancer cells. Cells were cultured in medium containing 5% charcoal-stripped fetal bovine serum for 6 days to deplete any endogenous steroids or effectors. Semi-confluent cells were then treated for 24 h with 2-ME₂ (1 nM - 10 μ M). Protein was extracted from the cells, quantified and subjected to SDS-PAGE and Western blot analysis. For functional analysis, alterations in T47D cell proliferation were quantified upon exposure to 2-ME₂. Laser scanning confocal microscopy was performed to determine the cytolocalization of p53 and ER α upon treatment with 2-ME₂. Western blot analysis revealed a relative increase in the level of p53 upon treatment with 5 nM - 10 μ M 2-ME₂ when compared to untreated controls. However, fluctuations were observed in the level of p53 upon treatment of cells with 100 nM - 10 μ M 2-ME₂. The presence of 100 nM - 10 μ M 2-ME₂ down-regulated ER α protein levels: at lower concentrations (1-10 nM), 2-ME₂ had no effect on ER α . Cell quantitation assay revealed a lack of significant effect of 1-10 nM 2-ME₂ on cell proliferation. However, a decrease (25 - 90%) in cell number was observed upon treatment with higher concentrations of 2-ME₂ (100 nM - 10 μ M). These observed effects on cell proliferation and regulation of p53 and ER α by 2-ME₂ may aid in further understanding the relationship between effects of hormone metabolites, steroid receptors, and tumor suppressor proteins in breast cancer cells.

Nothing to Disclose: AES, ALS, SD, VKM

Pub # P1-64

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Estrogen Receptor Subtype Expression Mediates Breast Cancer Risk Via Breast Epithelial Cell Proliferation

Author String K Britt, Y Razavi, S Hawthorne, G Risbridger
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Body Breast cancer (BC) risk is affected by a woman's exposure to estrogen. The increased levels of estrogen experienced at pregnancy reduce the risk of breast cancer in parous women by 50%. Unequivocally estrogens control of breast epithelial cell proliferation, but whether or not estrogen exposure alters the proliferative ability of the breast and thus BC risk, is unknown. The proliferative effects of estrogen are mediated via the estrogen alpha ($ER\alpha$) and counteracted by the anti-proliferative $ER\beta$ subtype. Both ERs are expressed in the normal breast epithelium but it is not known how the balance of these two receptors mediates epithelial proliferation, and thus BC risk. Stereological quantification of $ER\alpha$ expression showed region-specific expression that correlated with proliferation. In the mammary glands of parous mice, $ER\alpha$ was decreased by 31%, 42, and 49% in the proximal, central and distal regions respectively (distal region $p=0.03$) compared to nulliparous. $ER\beta$ is ubiquitously expressed in the gland however its region specific expression intensity was inversely correlated with proliferation. Parous glands showed increased $ER\beta$ in the distal regions and a more nuclear sub-cellular localisation. This was correlated with the expression of anti-proliferative marker FOXM1. Finally ER expression correlated with functional proliferative activity as assessed in vivo in estrogen-treated ovariectomised animals. It is concluded that ER subtype expression pattern in the normal breast dictates the proliferative nature of the gland and estrogen exposure modulates breast cancer risk, in part, by altering this balance.

Nothing to Disclose: KB, YR, SH, GR

Pub # P1-65

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Growth Hormone Potentiates Estrogen Action in Spontaneous Dwarf Rat Mammary Gland and in T47D Human Breast Cancer Cells

Author String DL Felice, S Zhao, M Spoula, C Kim, Q Shen, D Lantvit, TG Unterman, SM Swanson, J Frasor
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Body Estrogen (E2) is a major driving force in pubertal development of the mammary gland (MG) yet has little effect in the absence of pituitary hormones. Studies in hypophysectomized rodents suggest growth hormone (GH) may be the pituitary hormone critical for E2 action, but such animals lack all pituitary-derived hormone and have many endocrine deficits. Therefore, to examine specifically how GH and E2 interact we used ovariectomized Spontaneous Dwarf Rats (SDR) that lack circulating GH due to a mutation in the GH gene, but are otherwise genetically identical to the Sprague Dawley Rat. 5-day GH+E2 treatment of SDR resulted in more extensive MG ductal development and epithelial cell proliferation, shown by Ki67 expression, over E2 alone, indicating that GH potentiates the effects of E2. Furthermore, GH upregulated estrogen receptor α (ER α) mRNA and protein and potentiated E2-induced expression of the known ER target genes PR, ABCG2 and AREG. To examine if GH has a similar effect in breast cancer cells, we investigated GH+E2 crosstalk in the ER and GH receptor positive cell line, T47D. Indeed, 5-day GH+E2 treatment resulted in increased T47D cells compared to either hormone alone. GH did not affect ER α expression in T47D cells, yet still potentiated E2-induced transcription of the ER target genes PS2, AREG, and MYC. Interestingly, the time course of GH potentiation is distinctly different for each gene suggesting gene-specific mechanisms by which GH influence ER activity. For example, potentiation of AREG mRNA occurs only at later timepoints, due to increased transcription but not stabilization of the RNA. The exact mechanism by which GH potentiates E2 action on target genes is currently under further investigation. Many functions of GH are known to be mediated by both systemic and local IGF-I production but we found that GH did not affect IGF-I mRNA expression in T47D cells. Specific inhibition of IGF-I receptor (IGF-IR) activity reduced E2-stimulated proliferation. Yet, remarkably, GH bypassed the IGF-IR inhibition to potentiate E2-stimulated proliferation and target gene expression. The ability of GH to enhance E2-stimulated proliferation and gene expression in an IGF-I- and IGF-IR-independent manner has serious clinical implications for breast cancer patients who may be candidates for anti-IGF treatments. A better understanding of the GH/IGF/E2 axis may contribute to the development of novel drugs or improved approaches to therapy.

Nothing to Disclose: DLF, SZ, MS, CK, QS, DL, TGU, SMS, JF

Pub # P1-66

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Estradiol Induces Changes in the Expression and Localization of Tight Junction Proteins and HER-2, through the Activation of SRC in Human Breast Cancer Cell Line MCF-7

Author String JE Jimenez-Salazar, L Gonzalez-Nunez, A Luna-Lopez, M Konigsberg-Fainstein, LE Gomez-Quiroz, A Zentella-Dehesa, P Damian-Matsumura
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Body Tight junctions (TJ) play a key role in cell-to-cell adhesion in normal breast epithelium; however, their role in breast cancer progression and metastasis has been scarcely studied. This may be due to the lack of knowledge concerning the regulation on the expression and activity of the protein components of these junctions. This study sought to determine the effect of estradiol (E_2 ; $10^{-9}M$) on the expression and localization of TJ proteins including Zonula Occluden-1 (ZO-1), occludin, the ZO-1-associated transcription factor (ZONAB), on the activation of SRC (a protein-tyrosine kinase) and correlated to the expression of the epidermal growth factor receptor-2 (HER-2), in the human breast cancer cell line MCF-7.
We present data that demonstrate E_2 increases expression of ZO-1 and ZONAB mRNA (2-fold and 3.5-fold, after 15 min and 6 h of incubation, respectively) and protein (2.1-fold and 2.3-fold after 3 and 24 h of incubation, respectively); conversely, E_2 decreases significantly occludin mRNA (40%) and protein (50%) expression after 24 h of incubation. In order to determine if E_2 can induce translocation of ZO-1 and ZONAB proteins to the nucleus in the MCF-7 cells, immunolocalization assays were performed by Western blot hybridization of nuclear and cytoplasmic fractions and confirmed by confocal microscopy. Our results show that ZO-1 protein concentration decreased significantly in the cytoplasmic fraction after 30 min and, concomitantly, increased in the nuclear fraction after 60 min of E_2 -incubation. Similar results were observed with ZONAB protein, however higher concentrations of ZONAB were detected in the nuclear fraction in a shorter period. Both proteins were clearly co-localized in the nucleus by immunofluorescence microscopy after 30 min of E_2 -incubation. We suggest that changes in the localization of ZO-1 and ZONAB are associated to the activation of SRC, since active SRC increases after 30 min of incubation. Those results were correlated with the increase of HER-2 levels (3-fold) observed after 24 h of E_2 -incubation, effect that can be explained by the fact that ZO-1 and ZONAB are specific co-activator and transcription factor respectively in the promoter of the HER-2 gene.
All together, our results can explain the fact that E_2 is able to increase paracellular permeability and loss of cell-to-cell adhesion, enabling epithelial-mesenchymal transition of breast cancer cells.

Sources of Research Support: Grants from DCBS-UAM-I, CONACYT (66948-M) and Multidisciplinary project UAM (Rector-Agreement 13/2007). JEJ-S, LG-N and AL-P are recipients CONACYT's scholarships and enrolled in the Experimental Biology Graduate Program (212870), UAMI, MEXICO. We thank the support of the Laboratorio Divisional de Biología Molecular, UAM-I.

Nothing to Disclose: JEJ-S, LG-N, AL-L, MK-F, LEG-Q, AZ-D, PD-M

Pub # P1-67

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Elucidating the Role of HIF-1 α in Regulating Promoter II-Driven Aromatase Expression in Postmenopausal Breast Cancer

Author String NU Samarajeewa, ER Simpson, KA Brown
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Body The majority of postmenopausal breast cancers are dependent on estrogen synthesis. Tumor-derived factors such as prostaglandin E2 (PGE2) stimulate the CREB-dependent expression of aromatase promoter II (PII) via cAMP response elements (CREs), leading to the increased biosynthesis of estrogens in human breast adipose stromal cells (ASCs). Hypoxia inducible factor 1 alpha (HIF-1 α) acts as a key mediator of hypoxic responses in tumors. PGE2 has been shown to stabilize HIF-1 α independent of oxygen availability in human colon and prostate cancer cells. We have identified the presence of a consensus HIF-1 α binding motif overlapping with the proximal CRE of aromatase PII. However, the regulation of aromatase expression by HIF-1 α in breast cancer has not been characterized. This study aimed to characterize the role of HIF-1 α in the activation of aromatase PII.

The subcellular localization of endogenously expressed HIF-1 α was examined in ASCs by immunofluorescence/confocal imaging after treatment with PGE2 and dimethylallyl glycine (DMOG), which stabilizes HIF-1 α under normal oxygen tension. Both PGE2 and DMOG resulted in nuclear accumulation of HIF-1 α . Total aromatase transcript expression was also increased with both PGE2 and DMOG treatments. Notably, there was no significant change in total HIF-1 α mRNA levels with DMOG treatment. ChIP analysis demonstrated that DMOG treatment resulted in increased binding of HIF-1 α to aromatase PII. Reporter assays were performed to assess the effect of forskolin (FSK) and phorbol ester (PMA), to mimic PGE2, and DMOG treatments on aromatase PII activity after co-transfection with CRT2, a CREB-coactivator known to increase aromatase PII activity. FSK/PMA treatment resulted in a significant increase in PII activity, whereas DMOG alone had no effect. Interestingly, treatment with both FSK/PMA and DMOG resulted in a further significant increase in PII activity. Hence, it is possible that HIF-1 α contributes to the maximal induction of aromatase PII in response to tumor-derived factors such as PGE2, but requires other transcription factors, including CREB. In conclusion, this study is the first to identify HIF-1 α as a modulator of PII-driven aromatase expression in postmenopausal breast cancer. Together with our on-going studies on the role of AMP Kinase in the regulation of breast aromatase, this work provides another link between dysregulated metabolism and breast cancer.

Sources of Research Support: Fac Med PG Research Scholarship awarded to NUS; Victorian Breast Cancer Research Consortium; NHMRC project grant 1005735 awarded to KAB.

Nothing to Disclose: NUS, ERS, KAB

Pub # P1-68

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title A New Potent and Non-Estrogenic Steroidal Inhibitor of 17 β -Hydroxysteroid Dehydrogenase Type 1 Inhibits the Formation of Estrogen Estradiol *In Vitro* and *In Vivo*

Author String DP Ayan, R Maltais, J Roy, D Poirier
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Body 17 β -Hydroxysteroid dehydrogenase type 1 (17 β -HSD1) converts estrone (E1) into estradiol (E2) and is expressed in many steroidogenic tissues and breast cancer cell lines. Since the potent estrogen E2 stimulates the growth and development of hormone-dependent diseases, inhibition of the final step of E2 synthesis is considered a promising strategy for the treatment of breast cancer. Based on our previous study identifying 16 β -(*m*-carbamoylbenzyl)-E2 (CC-156) as a lead compound for the inhibition of 17 β -HSD1, we performed a number of structural modifications to reduce its undesired residual estrogenic activity. In order to test the inhibitory potency of the new compounds, we used the human breast cancer cell line T-47D, which exerts a strong endogenous 17 β -HSD1 activity. The steroid derivative PBRM (3-bromoethyl-16 β -(*m*-carbamoylbenzyl)-17 β -hydroxy-1,3,5(10)-estratriene) emerged as a potent inhibitor with an IC₅₀ value of 68 nM for the transformation of [¹⁴C]-E1 (60 nM) into [¹⁴C]-E2. When tested in two estrogen-sensitive breast cancer cell lines (MCF-7 and T-47D), PBRM demonstrated no proliferative (estrogenic) activity in the range of concentrations tested (0.01-10 [micro]M). Furthermore, with the purpose of evaluating the bioavailability of PBRM and CC-156 injected subcutaneously, we measured their plasmatic concentrations as a function of time, calculated the area under the curve (AUC) and demonstrated a significant improvement for PBRM (882 ng/ml*h) compared to CC-156 (445 ng/ml*h). We next tested the *in vivo* efficacy of PBRM on the T-47D xenograft tumor model, in female ovariectomized athymic nude mice. After 32 days of treatment with PBRM, tumor sizes in mice treated with E1 were completely reduced at the control group level (without E1 treatment). As a conclusion, PBRM is a promising 17 β -HSD1 inhibitor for the treatment of breast cancer, and could be used alternately or sequentially with other drugs against estrogen-dependent diseases.

Nothing to Disclose: DPA, RM, JR, DP

Pub # P1-69

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Progesterone Signaling in Hormone-Induced Mammary Tumors in ACI Rat

Author String AY Kariagina, J Xie, RC Opreanu, RN Horowitz, MD Basson, SZ Haslam
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Body Progesterone (P) are implicated in increasing risk for more invasive and more deadly breast cancers in postmenopausal women receiving hormone replacement therapy (HRT) with estrogen (E) plus P compared to women receiving E alone. To gain a better understanding of P action in breast cancer we investigated its role in development of hormone-induced mammary tumors in the rat. ACI rats were ovariectomized and treated with exogenous E or E+P. E-treated rats developed numerous foci of atypical hyperplasia (AH) that did not progress to ductal carcinoma in situ (DCIS) or tumors. In contrast, E+P treated rats developed a full range of mammary lesions, from AH to DCIS to invasive adenocarcinoma. Tumor development was associated with increased mRNA expression of progesterone receptor (PR) A and B, increased PRA and PRB protein expression, and an increase of the ratio of PRB:PRA. E+P-treated tumors exhibited increased proliferation of both luminal and basal/myoepithelial tumor cells compared to adjacent normal mammary glands, AH, or DCIS. Tumor cell proliferation was associated with increased cyclin E1 levels and redistribution of a cell cycle inhibitor, p27, from the nucleus to the cytoplasm. About 70% of E+P-treated tumors exhibited a decrease or loss of E-cadherin expression and were highly invasive. Tumor development in E+P-treated rats was accompanied with increased expression of a P-dependent paracrine factor, receptor activator of nuclear factor kappa B ligand (RANKL), and increased signaling downstream of RANKL. Consistent with the findings in the rat, RANKL was strongly induced *in vitro* by E+P treatment in human T47D breast cancer cells. In primary ER+PR+ human breast cancers, RANKL expression was detected in the majority of tumors from premenopausal women, who likely produce endogenous E and P. In contrast, RANKL was rarely expressed in tumors from postmenopausal patients. Our data suggest that in the ACI rat E alone is sufficient to promote transformation events in susceptible populations of cells and produce precancerous lesions, whereas P is additionally required for progression of transformed cells from precancerous lesions to frank tumors. These results raise the possibility that in women with hormone-dependent breast cancers, in addition to conventional anti-E treatments, the therapies targeting P signaling may provide a novel therapeutic strategy for tumors in premenopausal women who produce endogenous P or in postmenopausal women receiving E+P HRT.

Sources of Research Support: U.S. Army Medical Research and Materiel Command under W81XWH-07-1-0502 (to S.Z.H) and by the Breast Cancer and the Environment Research Grants 1U01 ES 012800 and 1U01 ES019434 from the National Institute of Environment Health Science (NIEHS) and the National Cancer Institute (NCI), National Institutes of Health, Department of Health and Human Services (to S.Z.H.). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS or NCI, NIH.

Nothing to Disclose: AYK, JX, RCO, RNH, MDB, SZH

Pub # P1-70

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Estrogen Metabolism and Risk of Breast Cancer in Postmenopausal Women

Author String BJ Fuhrman, C Schairer, MH Gail, J Boyd-Morin, X Xu, LY Sue, SS Buys, C Isaacs, LK Keefer, TD Veenstra, CD Berg, RN Hoover, RG Ziegler
National Cancer Institute, Bethesda, MD; Information Management Services, Inc, Silver Spring, MD; SAIC-Frederick, Inc, Frederick, MD; University of Utah Health Sciences Center, Salt Lake City, UT; Georgetown University, Washington, DC; National Cancer Institute at Frederick, Frederick, MD; National Cancer Institute, Bethesda, MD

Body Background: Estrogen is recognized as a causal factor in breast cancer. Estrone and estradiol, the parent estrogens, are irreversibly hydroxylated at the 2-, 4-, or 16-positions of the steroid ring. The mechanisms underlying estrogen-induced carcinogenesis and the role of estrogen metabolism have not been adequately evaluated in epidemiologic studies due to limitations of the assays available for measuring estrogens and estrogen metabolites (jointly referred to as EM).

Methods: We used a new liquid chromatography-tandem mass spectrometry method to assay 15 EM, in both total (sulfated + glucuronidated + unconjugated) and unconjugated forms, in prospectively stored serum from 277 women who developed invasive breast cancer and 423 matched controls. Subjects were drawn from women aged 55-74, not currently using hormone therapy, who were randomized to the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO). We calculated hazard ratios (HR) approximating risk in the highest, versus lowest, deciles of individual EM concentrations, EM grouped by metabolic pathway, and EM ratios using multivariate Cox proportional hazards models.

Results: Nearly all individual EM and EM groups were associated with increased breast cancer risk. Of the parent estrogens, unconjugated estradiol was the most strongly associated with risk [HR=2.07, 95% confidence interval (CI)=1.19 to 3.62, P trend (continuous)=0.01]. No individual EM or EM group remained significantly associated with risk after adjusting for unconjugated estradiol; however, the risk for unconjugated estradiol was also substantially attenuated in most of these models. After adjustment for unconjugated estradiol, two ratios of estrogen metabolism pathways, 2-hydroxylation pathway EM to parent estrogens and 4-hydroxylation pathway catechols to methylated catechols, were associated with significantly reduced [HR=0.72, 95% CI=0.52 to 1.00, P trend (continuous)=0.05] and significantly increased [HR=1.31, 95% CI=1.03 to 1.68, P trend (continuous)=0.03] breast cancer risk, respectively.

Conclusions: The extent of 2-hydroxylation of parent estrogens and of methylation of the potentially genotoxic catechol estrogens may, in addition to unconjugated estradiol concentrations, determine postmenopausal breast cancer risk.

Nothing to Disclose: BJF, CS, MHG, JB-M, XX, LYS, SSB, CI, LKK, TDV, CDB, RNH, RGZ

Pub # P1-71

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Curcuminoids Decrease Osteolytic Breast Cancer Bone Metastases

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Body Curcuminoids are polyphenolic compounds isolated from the rhizome of turmeric (*Curcuma longa*, L.), a botanical commonly used in Ayurvedic medicine. We previously found that curcuminoid-containing turmeric extracts inhibited osteoclast-mediated bone resorption in a translational model of rheumatoid arthritis and prevented rheumatoid synoviocyte expression of parathyroid hormone-related protein (PTHrP), a bone-resorptive peptide in rheumatic joints. Because PTHrP is also a critical breast cancer-derived mediator of lytic bone metastases, curcuminoid effects on osteolytic breast cancer bone metastases was tested. Four-week old female nude mice (n=11/group) were inoculated with MDA-MB-231 cells (1×10^5) into the left cardiac ventricle and treated ip every other day with a curcuminoid-enriched extract (50 mg curcuminoids/kg/dose) or vehicle. Evidence of lytic bone lesions in the hind limbs was evaluated prospectively for 3 weeks by x-ray. Curcuminoid-treated mice had significantly less osteolytic bone lesion area compared to vehicle-treated mice ($2.0 \pm 1.0 \text{ mm}^2$ vs. $11.1 \pm 2.1 \text{ mm}^2$; $p < 0.05$). *In vitro*, when MDA-MB-231 cells were pre-treated with curcuminoids (0.3-10 $\mu\text{g/ml}$) and stimulated with TGF- β (5 ng/ml) for 24 hours, PTHrP secretion, as measured by radioimmunoassay, was inhibited by curcuminoids ($\text{IC}_{50} = 2.1 \mu\text{g}$) independent of effects on cell proliferation and survival. These initial data suggest that curcuminoids may decrease lytic bone metastases in breast cancer through inhibition of breast cancer cell expression of PTHrP.

Sources of Research Support: NIH Grant AT004875 awarded to JLF.

Nothing to Disclose: LEW, TAG, KSM, JLF

Pub # P1-72

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Investigating Agents That Invoke Apoptotic Synergy of Long-Term Estrogen-Deprived Breast Cancer Cells

Author String SE Aiyar, W Wang, J-p Wang, W Yue, RJ Santen
University of Virginia, Charlottesville, VA

Body

Background: Acquired resistance to estrogen lowering agents such as aromatase inhibitors (AIs) or tamoxifen occurs in a substantial number of postmenopausal women. Resistance can occur in part due to upregulation in growth factor pathways (HER2, mTOR) or it can be the result of blocks in the cell death pathways (c-FLIP, Bcl-2). We hypothesize the best way to avoid resistance is to kill the tumor cells before they have a chance to adapt. Therefore, we are testing a combination of agents which either target the death receptor or intrinsic mitochondrial pathways.

Materials & Methods: Our in vitro system utilizes breast cancer cell lines that have been deprived of estradiol long term. We are using 4-(4-Chloro-2-methylphenoxy)-N-hydroxybutanamide, (CMH) since this agent sensitizes resistant cells to stimuli of the extrinsic death receptor pathway of caspase activation, including FAS, TRAIL and anoikis. CMH is a small molecule inhibitor of c-FLIP as well as HDACs 3, 6 and 8. To target the intrinsic mitochondrial death pathway we are using: Salirasib (FTS) and E2. FTS (Salirasib), inhibits ras and mTOR, it reduces survivin, dephosphorylates Bad, activates Bax and caspases which in turn activate the mitochondrial death pathway. Estrogen can induce apoptosis of Long-term estrogen deprived cultures by increasing Bax and Bim and activating of Caspase-7.

We are using the CompuSyn program to generate isobolograms and to determine the combination index (CI). The combination index can quantitatively depict synergism (CI <1), an additive effect (CI = 1), and antagonism (CI >1).

Results: We found that the combination of FTS (Salirasib) with CMH resulted in a synergistic interaction where effective doses 50, 75, and 90 ranged from 0.811-0.719. The combination of FTS and estradiol also resulted in a synergistic interaction where effective doses 50, 75 and 90 ranged from 0.635 to 0.813. We did not find synergy with a combination of estradiol and CMH.

Conclusion: Drug combination analysis is useful for reducing toxicity and resistance. We have found that the combinations of CMH and Salirasib as well as the combination of Sailrasib and estradiol displayed synergy. Our future directions are to test the synergistic combinations in the mouse LTED xenograft model.

Sources of Research Support: Susan G. Komen foundation (KG080267).

Nothing to Disclose: SEA, WW, J-PW, WY, RJS

Pub # P1-73

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Association between Premenopausal Circulating Sex Steroids and Breast Cancer Risk

Author String RT Fortner, AH Eliassen, SE Hankinson
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Body Prior research supports an association between endogenous sex steroids and breast cancer among postmenopausal women; however, the association is less clear among premenopausal women. Limited prior research, including our own, supports an association between premenopausal endogenous estrogens and androgens and breast cancer risk. We evaluated the association between estrogens, androgens, progesterone, and sex hormone binding globulin (SHBG) and breast cancer in a nested case-control study in the Nurses' Health Study II. Between 1996-1999, 29,611 participants provided blood samples; 18,521 provided samples timed in early follicular and mid-luteal phases of the menstrual cycle. 482 of these women developed breast cancer between 1999 and 2007 (386 timed samples); these women were matched to 965 controls (777 timed samples). Estrogens and androgens were assayed in two groups, one by radioimmunoassay and one by liquid chromatography-tandem mass spectrometry; progesterone, SHBG, and DHEAS were measured with chemiluminescence immunoassay. We used conditional logistic regression controlling for breast cancer risk factors. We found no association between estrogens, testosterone, progesterone or SHBG and breast cancer risk when all cancers were considered, with the suggestion of increased risk with higher DHEAS (RR range: luteal estrone, 0.8, 95% CI: 0.6-1.2, $p_{trend}=0.38$ to DHEAS, 1.3, 95% CI: 1.0-1.7, $p_{trend}=0.55$; 4th vs. 1st quartile). Evaluating ER+/PR+ tumors, increased risk was associated with higher luteal estradiol (RR: 1.7, 95% CI: 1.0-3.1, $p_{trend}=0.13$), testosterone (RR: 1.7, 95% CI: 1.0-2.9, $p_{trend}=0.09$), DHEAS (RR: 1.5, 95% CI: 1.0-2.3, $p_{trend}=0.35$), luteal free estradiol (RR: 1.8, 95%: 1.1-3.1, $p_{trend}=0.14$) and free testosterone (RR: 1.4, 95% CI: 1.0-2.1, $p_{trend}=0.25$). Among women diagnosed with premenopausal breast cancer, higher follicular estradiol was associated with invasive cancer (RR: 1.7, 95% CI: 1.0-2.8, $p_{trend}=0.35$). Among women diagnosed after menopause, DHEAS was associated with breast cancer risk (RR: 4.5, 95% CI: 1.4-14.6; $p_{trend}<0.01$), though power was limited in this subgroup. Our findings suggest no association between premenopausal estrogens, progesterone, and SHBG and overall breast cancer risk, with associations between estrogens and androgens and risk of ER+/PR+ breast cancer. Follicular estradiol may also be associated with premenopausal invasive breast cancer and DHEAS associated with both pre- and postmenopausal breast cancers.

Sources of Research Support: NIH CA067262; NIH CA050385; NIH T32 CA09001 (RT Fortner).

Nothing to Disclose: RTF, AHE, SEH

Pub # P1-74

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Differential Gene Regulation by Estradiol, Estriol and Estetrol Treatment in Breast Cancer Cells

Author String M Laine, SS Rajan, K Vanek, AL Zarnke, GL Greene
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Body Estetrol (E4) and Estriol (E3) are estrogen derivatives that are particularly abundant during pregnancy and are found at high concentrations in maternal plasma and the fetus. E4 has been extensively studied as a potential treatment to prevent menopausal symptoms, and has been shown to have a beneficial effect in the treatment of osteoporosis. Multiple studies suggest that E4 is a good candidate for hormone replacement therapy (HRT). While the effects of E3 on breast cancer are less clear, E4 has been shown to reduce tumor size and number in a rat breast cancer model. E4 is postulated to have an antagonist effect in the presence of Estradiol (E2). Although E4 and E3 bind to the Estrogen Receptor alpha (ER α) with a weaker affinity than E2, they have a long half-life in vivo and significant bioavailability when administered orally. Therefore molecular and genomic studies of E2, E3, E4 should help elucidate the mechanisms by which these 3 steroids vary in their potencies and effects on breast cancer.

We have determined the crystal structures of the ligand binding domain (LBD) of ER α in complex with E3 (PDB: 3Q95) and E4 (PDB: 3L03) to provide a structural context for the observed differences. The Y537S mutant LBD adopts an agonist conformation in both complexes. As a consequence of the additional hydroxyl groups in E3 and E4, helix 8 is slightly displaced relative to the E2 structure (PDB: 1ERE). While both E3 and E4 retain all the interactions with the protein as seen with E2, the E3 forms weaker hydrogen bonds with H524 compared to E2.

A PCR array analysis of human breast cancer pathways, performed on MCF7 cells treated with E2, E3 or E4, revealed differential responses to these ER ligands. This array includes genes directly associated with breast cancer as well as genes involved in estrogen-dependent signaling. Our results show that several genes involved in immune response, inflammation/cytokine response, as well as oncogene and cell proliferation markers, are differentially regulated by E2, E3 and E4 in MCF7 cells. We are currently investigating the role of both ER in different breast cancer cell lines as potential factors in the differential regulations of these genes. The results of this study will provide a better understanding of the effects induced by natural estrogen metabolites and may contribute to new hormone replacement strategies that reduce the risk of breast cancer while also reducing many of the undesirable effects of current HRT agents.

Nothing to Disclose: ML, SSR, KV, ALZ, GLG

Pub # P1-75

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Body Mass Index and Risk of Death among Breast Cancer Survivors: The California Teachers Study

Author String CM Dieli-Conwright, L Duan, H Ma, J Sullivan-Halley, P Reynolds, S Neuhausen, L Bernstein, KD Henderson
Beckman Research Institute at the City of Hope National Medical Center, Duarte, CA; University of Southern California, Los Angeles, CA; Cancer Prevention Institute of California, Fremont, CA

Body Obesity has been reported to increase risk of death among breast cancer survivors. Few studies have examined this effect in relation to disease stage and estrogen receptor status. We evaluated these effects among participants in the California Teachers Study.

Methods
The analysis included 3,995 participants in the California Teachers Study cohort who were diagnosed with invasive breast cancer between 1995 and 2006. 583 total deaths accrued through 2007, including 262 breast cancer deaths and 321 non-breast cancer deaths. Height and weight were self-reported on the baseline questionnaire, as was weight at age 18. Baseline body mass index (BMI) and change in BMI from age 18 to baseline were calculated. Cox proportional hazards models were used to evaluate associations between BMI measures and risk of breast cancer mortality and risk of all-cause mortality, stratified by disease stage and estrogen receptor status.

Results
Obesity (BMI [ge] 30 kg/m²) at baseline was statistically significantly associated with increased breast cancer mortality risk (RR 1.69, 95% CI 1.20-2.38). Obesity at baseline (BMI [ge] 30kg/m²) was strongly associated with breast cancer mortality risk (RR 1.91, 95% CI 1.27-2.87) among participants diagnosed with regional or distant disease; there was no association of obesity and mortality among those with localized disease. Higher baseline BMI was associated with higher breast cancer mortality risk among participants with estrogen receptor positive (p for trend = 0.04) but not estrogen receptor negative disease (p for trend = 0.41). BMI at age 18 between 25 and 29 kg/m² was statistically significantly associated with increased breast cancer mortality risk (RR 1.69, 95% CI 1.09-2.61). BMI at age 18 between 25 and 29 kg/m² was statistically significantly associated with increased breast cancer mortality risk among participants with estrogen receptor negative breast cancer (RR 3.23, 95% CI 1.29-8.07). Results for analyses of all-cause mortality risk were similar, and somewhat attenuated from those reported for breast cancer mortality risk.

Conclusions
These findings suggest that obesity, throughout a woman's life, may play an important role in mortality risk among women diagnosed with invasive breast cancer, providing further justification for utilizing public health strategies to decrease and prevent obesity throughout the lifespan.

Sources of Research Support: Grant R01 CA77398 from the National Cancer Institute, and contract 97-10500 from the California Breast Cancer Research Fund.

Nothing to Disclose: CMD-C, LD, HM, JS-H, PR, SN, LB, KDH

Pub # P1-76
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)
Title Hyperinsulinemia Is Associated with Increased Incidence of Pulmonary Metastasis of Mammary Tumor Cells in an MKR Mouse Model of Type 2 Diabetes
Author String RD Ferguson, R Novosyadlyy, Y Fierz, H Sun, Y Shoshana, D Leroith
Mount Sinai School of Medicine, New York City, NY

Body It has been demonstrated that hyperinsulinemia, found in the early stages of T2D, can promote an increase in proliferation of target cells, mediated by the insulin receptor (IR) and /or insulin-like growth factor receptor 1 (IGFR-1). In this study we propose that the elevated insulin levels characteristic of T2D may promote metastasis of tumor cells from the primary site to distant organs such as the lungs. We use the hyperinsulinemic, non-obese, mildly hyperglycemic MKR female mouse model to investigate the effects of elevated circulating insulin levels on the frequency of mammary tumor metastasis to lung. At eight weeks of age female MKR or control mice were injected orthotopically with murine mammary tumor cell line Mvt1 (transformed with c-myc). When primary mammary tumors in both groups reached a volume of 40mm³ they were surgically removed and mice were followed up for additional 4 weeks. We found a significantly greater mean number of spontaneous metastases in MKR mice as compared to controls (MKR, 22 versus control, 5; P< 0.01). Additional groups of MKR and control mice were injected intravenously with identical numbers of Mvt1 cells and were sacrificed three weeks later. Using this model we also found that MKR mice had significantly higher mean number of metastases when compared to controls (MKR, 6.5 versus control 1.5; P<0.01). Overall, we conclude that hyperinsulinemia increases the number of metastatic lesions formed in the lungs independent of tumor size. Thus, we propose that the hyperinsulinemic conditions found in certain stages of T2D may advance the occurrence of metastases during breast cancer progression.

Nothing to Disclose: RDF, RN, YF, HS, YS, DL

Pub # P1-77

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Prolonged Hyperinsulinemia in the Immunodeficient MKR Mouse Model Leads to More Rapid Growth of Human Breast Cancer Cells and a Significant Enlargement of Thymus Tissue

Author String RD Ferguson, R Novosyadlyy, S Yakar, D Leroith
Mount Sinai School of Medicine, New York, NY

Body The recombination activating genes (RAG) - 1 and - 2 are critical for T-cell development and subsequent B-cell maturation. Mice deficient in either RAG-1 or RAG-2 are severely immunocompromised and thus provide a useful model to study tumor formation and progression initiated by human cancer cells. Several epidemiological studies have shown that the risk of human breast cancer development and progression may be linked to type 2 diabetes (T2D). One of the hallmarks of early stage T2D is severe insulin resistance, which results in chronic hyperinsulinemia. To investigate whether hyperinsulinemia affects human breast cancer cell growth *in vivo* we have crossed RAG1-deficient (RAG1^{-/-}) with MKR mice and generated a mouse model of hyperinsulinemia on an immunodeficient background. We found that the hyperinsulinemic environment in an immunodeficient background (MKR/RAG1^{-/-}) led to significant inflammation in immune organs, such as the thymus. When injected with luciferase-labeled human mammary tumor cells (MDA-MB-231-luc⁺), female MKR/RAG1^{-/-} mice (n=5) showed a consistently greater flux (photons/second/cell) during tumor growth than those in control mice (n=5) over a period of 24 weeks. Furthermore, at time of sacrifice, all MKR/RAG1^{-/-} mice exhibited a significantly enlarged thymus whereas no members of the RAG1^{-/-} control group showed this trait. We conclude that high systemic insulin levels *in vivo* lead to more rapid development of human mammary tumors initiated by orthotopically injected human breast cancer cells. Furthermore, hyperinsulinemia, possibly combined with the presence of human cancer cells, caused excessive inflammation of the thymus, which may have implications in tumor progression.

Nothing to Disclose: RDF, RN, SY, DL

Pub # P1-78

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Growth Hormone Enhances Breast Cancer Chemoresistance by Inhibiting JNK-Mediated Apoptosis

Author String M Minoia, E Gentilin, F Tagliati, D Mole, MR Ambrosio, A Baroni, MC Zatelli, E degli Uberti
Section of Endocrinology, University of Ferrara, Ferrara, Italy; Pfizer Italia srl, Roma, Italy

Body Growth hormone (GH) and insulin-like growth factor (IGF-I) are known to promote breast carcinogenesis. Breast cancer (BC) incidence is not increased in female acromegalic patients, but mortality is greater as compared to general population. We previously demonstrated that GH/IGF-I excess influences BC response to chemotherapy in vitro, possibly accounting for the increased mortality. We indeed showed that GH and IGF-I induce cell proliferation of a BC cell line, the MCF7 cells, providing protection towards the cytotoxic effects of doxorubicin (D). GH effects are direct and not mediated by IGF-I, since they are apparent also in the presence of an IGF-I receptor blocking antibody and disappear in the presence of the GH receptor antagonist Pegvisomant (Peg). The aim of the present study was to evaluate the possible mechanisms implicated in the protective action of GH towards the cytotoxic effects of D in the BC cell line, MCF7. We investigated the combined effects of GH and D on MCF7 cell cycle, apoptosis, glutathione-S transferase (GST) activity and JNK transcriptional activity. We found that GH does not revert D-induced accumulation of MCF7 cells in G2/M phase of the cell cycle. However, GH significantly reduced both basal and D-induced MCF7 cell apoptosis, an effect nearly completely blocked by Peg. We therefore explored the involvement of GST, which activation protects DNA against damage by anticancer drugs. However, GH treatment significantly reduced GST activity in MCF7 cells, as well as Peg, possibly indicating a greater susceptibility of Peg-treated cells to DNA damage. In addition, GH reduced basal and D-stimulated JNK transcriptional activity. These data altogether indicate that GH directly induces resistance to chemotherapeutic drugs by protecting the cells from apoptosis, at least in part through a reduction in JNK transcriptional activity. Our data further support the hypothesis that GH excess might hamper BC treatment, possibly resulting in an increased mortality.

Disclosures: AB: Medical Advisory Board Member, Pfizer, Inc. EdU: Principal Investigator, Pfizer, Inc.
Nothing to Disclose: MM, EG, FT, DM, MRA, MCZ

Pub # P1-79
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)
Title Evaluation of Changes in Mammographic Breast Density Associated with Bazedoxifene/Conjugated Estrogens in Postmenopausal Women
Author String JA Harvey, JV Pinkerton, EC Baracat, H Shi, S Mirkin, AA Chines
University of Virginia, Charlottesville, VA; University of Virginia, Charlottesville, VA; Universidade de São Paulo, São Paulo, Brazil; Pfizer Inc, Collegeville, PA

Body **Introduction:** The tissue selective estrogen complex (TSEC) pairs a selective estrogen receptor modulator (SERM) with 1 or more estrogens. A TSEC that pairs bazedoxifene (BZA) with conjugated estrogens (CE) has been shown to have a neutral effect on the breast in the phase 3 Selective estrogens, Menopause, And Response to Therapy (SMART) trials. The relationship between breast density and breast cancer risk is not well understood, but increased breast density can substantially reduce the sensitivity to detect abnormalities. SERMs, including BZA, have not been shown to increase breast density. This study evaluated the quantitative changes from baseline in mammographic breast density after 24 months of therapy with BZA 20 mg/CE 0.45 or 0.625 mg, raloxifene (RLX) 60 mg, or placebo in postmenopausal women.
Methods: This was an ancillary study of a 2-year, randomized, double-blind, placebo- and active-controlled phase 3 trial in postmenopausal women (SMART-1). The treatments evaluated in this ancillary study were BZA 20 mg/CE 0.45 and 0.625 mg, RLX 60 mg, and placebo. Eligible subjects must have completed 24 months of treatment and have mammograms at baseline and at 24 months. Original mammogram film pairs (left craniocaudal views) were digitized at a central imaging center, masked, and sent for evaluation to a single radiologist who was blinded to the time sequence of the images, treatment assignment, and all subject information. Percent breast density was determined using a previously validated software program.
Results: The mammograms from 507 subjects (mean age range, 55.2-56.3 y) were evaluated in this study. At Month 24, the mean (\pm standard deviation) percent change from baseline was similar with BZA 20 mg/CE 0.45 mg (-0.39% [1.75%]), BZA 20 mg/CE 0.625 mg (-0.05% [1.68%]), RLX 60 mg (-0.23% [1.76%]), and placebo (-0.42% [1.72%]). The reductions from baseline were statistically significant for BZA 20 mg/CE 0.45 mg and placebo (95% confidence intervals, -0.69 to -0.08 and -0.72 to -0.11, respectively). In a prespecified subgroup analysis, both BZA/CE doses had a generally consistent clinical effect on breast density among subgroups based on age, body mass index and years since last menstrual period.
Conclusions: Treatment with BZA 20 mg/CE 0.45 or 0.625 mg did not affect age-related changes in breast density over 2 years in this population of postmenopausal women.

Disclosures: JVP: Investigator, Pfizer, Inc.; Consultant, Pfizer, Inc.; Amgen; Teva; Novo Nordisk; Boeinger-Ingelheim; DepoMed. HS: Employee, Pfizer, Inc. SM: Employee, Pfizer, Inc. AAC: Employee, Pfizer, Inc. Nothing to Disclose: JAH, ECB

Pub # P1-80

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Gene Expression Profiles of *N*-Methyl-*N*-Nitrosourea (MNU)-Induced Mammary Cancers from Intact and Ovariectomized Rats

Author String AP Betancourt, C Lema, G Thordarson
University of Texas at El Paso, El Paso, TX

Body Most estrogen receptor (ER)-positive breast cancers respond initially well to hormone therapy, but usually relapse within months or a few years of treatment (1), giving rise to estrogen-independent tumors. Although over 90% of *N*-methyl-*N*-nitrosourea (MNU)-induced rat mammary cancers either become impalpable or show more than 50% reduction in size after ovariectomy, a high percentage of these tumors show re-growth with prolonged time after ovariectomy. (2), making this animal model appropriate to study the progression of estrogen-dependent breast cancer to estrogen independence. Here, this animal model was used to study the differences in the gene expression of mammary tumors from intact and ovariectomized (OVX) rats. Virgin Sprague-Dawley rats were injected with a single dose of MNU (50 mg/kg body wt) at 50-60 days of age. Mammary tumors that developed were allowed to grow to 1.5 cm in diameter. At that time, half of the animal were sacrificed and mammary tumors collected, but the remaining animals were ovariectomized. The regression and then regrowth of the tumors in the OVX animals was monitored and they were collected when they had reached 1.5 cm in diameter. Total RNA was extracted from the tumors and used for DNA microarray analysis. Among the more than 40,000 genes that were analyzed, 12,988 showed differential expression ([ge] 2-fold change) between the two groups of animals. Tumors obtained from intact rats showed higher expression of 8,418 genes whereas tumors from OVX animals showed higher expression of 4,570 genes. Comparison to homologous genes that have been identified as signature genes for different subtypes of human breast cancers (3) revealed that the expression profile of tumors from intact animals most resembled the Luminal A subtype, but tumors from OVX rats were most similar to the ERBB2 subtype. Studies are now underway on a subset of 3,326 genes differentially expressed (*p*-value [le]0.01 and [ge]4.0 fold change) to identify biological pathways differentially expressed in the two tumor type.

1. Normanno N et al., *Endocrine-Related Cancer* 2005; 12: 721
2. Thordarson G et al., 2001; *Carcinogenesis* 22: 2039
3. Sorlie T 2001; *PNAS* 98: 10869

Sources of Research Support: NIH/NCI grants RO3CA128067 and RO3CA128067-01A1S1 awarded to GT; NIH/NCRR grant 5G12RR008124-17 awarded to BBRC UTEP; the DNA Analysis Core Facilities at UTEP.

Nothing to Disclose: APB, CL, GT

Pub # P1-81

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Lack of PMCA2 Prevents Mammary Tumorigenesis in MMTV-Neu Mice

Author String JN VanHouten, CE Sullivan, JJ Wysolmerski
Yale Medical School, New Haven, CT; Yale Medical School, New Haven, CT

Body The plasma membrane calcium-ATPase, isoform 2 (PMCA2) is important for calcium transport into milk, and for maintaining the survival of mammary epithelial cells during lactation. At weaning, milk stasis causes rapid down-regulation of PMCA2 expression and intracellular calcium crisis that contributes to widespread apoptosis in early involution. In a breast cancer cell line, PMCA2 over-expression protected the cells from calcium-induced apoptosis. Tissue microarray analysis revealed that high PMCA2 expression correlated with decreased survival in breast cancer patients. Therefore, we hypothesized that PMCA2 might contribute to mammary tumor development and/or progression. In the current study, we examine the effect of the absence of PMCA2 in a mouse model of breast cancer by crossing dfw-2J, a PMCA2-null mutant, and MMTV-Neu mice. Our results suggest that PMCA2 is necessary for Her2/Neu/ErbB2-induced mammary tumorigenesis, because only 1 of 10 MMTV-Neu/dfw-2J mice developed a tumor, compared to 8 of 13 MMTV-Neu controls. PMCA2 appears to be necessary early in tumorigenesis, since hyperplasias were uncommon in mammary glands of MMTV-Neu/dfw-2J mice, despite robust expression of the Neu transgene. Further tissue microarray analysis revealed an interaction between PMCA2 and Her2 levels in predicting breast cancer patient survival. We conclude that PMCA2 may be fundamentally important for the development of Her2/Neu/ErbB2 positive breast cancer, and targeting PMCA2 may improve existing therapies directed at the Her2/Neu/ErbB2 pathway

Sources of Research Support: Susan G. Komen grant KG081429 to JVH; NIH grant DK069542 to JW.

Nothing to Disclose: JNV, CES, JJW

Pub # P1-82

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Prevalence of Familial Diabetes Differs between Type 2 Diabetes Mellitus Patients with Cancer and Those without Cancer

Author String LM Berstein, MP Boyarkina, SY Teslenko
NN Petrov Research Institute Oncology, St Petersburg, Russian Federation; LG Sokolov Clinical Hospital, St Petersburg, Russian Federation

Body Objectives: Since type 2 diabetes mellitus (DM2) is a risk factor of a number of malignancies, it is important to find out what factors can modify this association. Family history of DM2 is known to be associated with increased risk of diabetes. Many cancers also show family clustering, which is not always genetic. However, strange as it is, the available literature related to a role of familial DM2 as a modifier of cancer risk is rather scarce and ambiguous. Materials: The present case-control study was designed to assess DM2 prevalence among the parents or siblings of cancer patients who (a) did not have (n=77; age 59.3±1.3 years) or (b) had overt (n=197; 63.7±0.6 years) or latent (n=25; 61.5±1.5 years) DM2 and (c) of DM2 patients without cancer (n=222; 62.0±0.7 years). Results: In the kin of cancer-free diabetics, DM2 was found to be significantly more prevalent (30.6±3.2%, p < 0.01) than in the families of DM2 patients having mammary (9.5±4.5%), endometrial (10.8±5.1%) or any cancer (in all 15.8±2.4%; in female patients 14.8±2.8%) and of cancer patients without DM2 (in whole group of patients 6.5±2.8%; in female patients 5.0±3.4%). In DM2 patients with cancer, the prevalence of parents with DM2 was the same (about 75% of all kin) as in cancer-free diabetics; therefore, there are no grounds to suspect any changes in the parent/sibling ratio. Body mass index and gender also were not among the factors explaining revealed distinctions. On the other hand, DM2 patients without cancer who had parents or siblings having DM2 featured preference for use of biguanide metformin vs. sulfonylurea derivatives in comparison with mammary or endometrial cancer patients, either with or without family history of DM2. Conclusions: Thus, although it is difficult to determine by present whether genetics, behavior or environmental factors play the major role in mediating notably weaker associations with familial diabetes in cancer patients than in diabetics without cancer, it can not be excluded that type of anti-diabetes therapy may be a factor of influence and deserves further study.

Nothing to Disclose: LMB, MPB, SYT

Pub # P1-83

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Prolactin Promotes Breast Cancer Cell Chemoresistance in a Mechanism That Uses HSP90

Author String A Urbanska, O Karayazi, F Boutillon, V Goffin, CS Shemanko
University of Calgary, Calgary, Canada; University Paris Descartes, Paris, France

Body We have made an observation that the hormone prolactin mediates chemotherapeutic resistance to DNA damaging agents in breast cancer cells in a mechanism that involves heat shock protein 90 (HSP90). Prolactin is a hormone secreted by the pituitary gland, as well as by normal breast cells and some breast cancers. High serum prolactin levels are associated with increased incidence of rodent mammary tumors, increased risk of breast cancer in humans and are associated with an overall worse survival in breast cancer patients and cancer progression. Recently it was reported to increase resistance in vitro to a variety of chemotherapeutics that have different mechanisms of action and therefore, likely induce different mechanisms of resistance.

With the interest in elucidating the role of prolactin in cancer, we were the first to use a large-scale screen to identify new prolactin-regulated genes in breast cancer cells (1). We discovered that HSP90a, known as the master cancer chaperone, is a prolactin regulated gene and that both prolactin and HSP90a promote cell survival. HSP90a acts as a protein chaperone for cellular signaling molecules involved in cancer, and binds to these substrate proteins, known as client proteins. Our unpublished observations identify a HSP90-based mechanism by which prolactin increases resistance to DNA damaging anti-cancer agents in breast cancer cells. We observed in both estrogen receptor-positive (MCF7) and estrogen receptor-negative cells (SKBR3), that prolactin increased cell viability of either doxorubicin- or etoposide-treated cells. The effect was specific to the prolactin receptor, as a pure prolactin receptor antagonist (del1-9-G129R-hPRL) abolished the prolactin-mediated increase in survival of the drug treated cells. We identified a role for HSP90 in prolactin-mediated resistance to these DNA damaging agents, as the prolactin-mediated increase in cell viability was abolished in the presence of a specific HSP90 inhibitor. Therefore, we hypothesize that prolactin induces resistance to DNA damaging agents, and that this is mediated via HSP90. We also have identified a candidate client protein that depends upon HSP90 for stability that may be important in this mechanism. Understanding the mechanisms of chemoresistance is key to better therapeutic strategies. HSP90 inhibitors are already in clinical trial, although we may provide support to combine them with prolactin receptor antagonists which are in preclinical trials.

(1) Perotti et al 2008 Breast Cancer Research 10, R94

Nothing to Disclose: AU, OK, FB, VG, CSS

Pub # P1-84

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title Defining Complexities in the Responses of Breast Cancer Cells to Low and High Doses of Progestins: Less Is More?

Author String H Wade, D McDonnell, M Brown
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Body Progesterone is a naturally occurring steroid hormone that functions by binding to the progesterone receptor (PR) and thereby enabling the receptor to bind DNA, recruit cofactors, and induce the transcription of target genes. In the breast and other tissues of the female reproductive system, progesterone plays an important role in normal development and function. Therefore, synthetic progestins are widely used to manipulate the downstream biology of PR for purposes including contraception and hormone replacement therapy. However, progestins and PR have also been implicated in disease pathologies such as breast cancer. While the molecular mechanisms by which PR regulates breast tumor growth and metastasis have not been fully elucidated, recent studies highlight the fact that PR has a complex role in breast cancer progression that may be dependent on ligand dose. For example, data from the WHI trial suggest that low-dose progestins used in combination with estrogen are associated with an increased risk of invasive breast cancer compared to estrogen alone. On the other hand, high-dose progestin therapy has been used as an effective front-line and second-line treatment for advanced, metastatic breast cancer. To investigate this paradox, we undertook studies to identify aspects of PR biology that respond differently to treatment with low or high doses of progestins. Interestingly, we found that treatment of T47D breast cancer cells with low-dose progestins generates a significantly more robust induction of a subset of PR target genes, including the cell cycle regulators cyclin D1 and E2F1, than treatment with high-dose progestins. Furthermore, we show that varying doses of progestins have differential effects on the receptor itself, including the subcellular localization, phosphorylation and subsequent turnover of PR. At the level of transcriptional regulation, our ChIP analyses have led us to hypothesize that the strength of PR activation of target gene expression may hinge upon the ratio of phospho-PR versus unmodified PR that is recruited to enhancer elements. This dose-dependent effect may result in the formation of different PR transcriptional complexes and explain the diverse downstream biologies that are regulated by varying levels of progestins throughout the female reproductive cycle and in diseases such as breast cancer. Given the wide range of medical therapies that utilize progestins, our findings may have important clinical implications.

Nothing to Disclose: HW, DM, MB

Pub # P1-85

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Hormones & Breast Cancer (1:30 PM-3:30 PM)

Title The Omega-3 Fatty Acid EPA Does Not Inhibit Progestin Stimulation of *In Vitro* Invasive Properties in T47D Human Breast Cancer Cells

Author String RA King, MR Moore
Joan C Edwards School of Medicine, Marshall University, Huntington, WV

Body Clinical studies have shown that progestins increase breast cancer risk when included in hormone replacement therapy. We and others have previously reported that progestins also stimulate invasive properties in the progesterone receptor-rich human breast cancer cell line T47D. Invasive properties are required for metastasis and since metastasis is what causes death from cancer, it is important to find effective ways to prevent metastasis. Other researchers have reported that omega-3 fatty acids inhibit metastatic properties of other human breast cancer cell lines both in vitro and in vivo. We wanted to test the hypothesis that the omega-3 fatty acid EPA would inhibit progestin stimulation of invasive properties, and thus possibly be effective in combating metastasis of progestin-responsive breast cancer. T47D cells were grown in minimal essential medium, with 10% fetal bovine serum. The medium was then changed to fresh and made 1-200 micromolar in EPA (omega-3) (eicosapentaenoic acid), AA (omega-6)(arachidonic acid), or 0.1% in vehicle (ethanol) , so that ethanol concentration in all cultures was 0.1%. The cells were then incubated at 37 degrees for 72 hrs, and made 10 micromolar in ara-C for the last hour of incubation. The cells were then harvested, single cell suspensions made, and incubated for 48 hrs in modified Boyden chambers to measure invasion through extracellular matrix and a porous membrane, with and without the synthetic progestin R5020 (10 nM) in the same medium as above (including ara-C), except without phenol red and serum, for 48 hrs. The invading cells were then counted. Surprisingly, neither EPA nor AA inhibited progestin stimulation of invasive properties, and, at 200 micromolar, EPA alone stimulated invasion. The omega-3 fatty acid EPA, at 200 micromolar, does not inhibit progestin stimulation of in vitro invasive properties in T47D human breast cancer cells, but by itself stimulates invasion.

Nothing to Disclose: RAK, MRM

Pub # P1-86
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)
Title Gold Nanoparticles Do Not Affect Ovarian Inhibin Accumulation *In Vitro*
Author String JK Larson, G Watanabe, K Taya, MJ Carvan III, R Klaper, RJ Hutz
University of Wisconsin-Milwaukee (UWM), Milwaukee, WI; UWM, Milwaukee, WI; UWM and Children's Research Institute (CRI), Milwaukee, WI; Tokyo University of Agriculture and Technology (TUAT), Fuchu, Japan

Body Gold nanoparticles (GNPs) have gained considerable attention for use in medicinal drug delivery, bio-imaging, and diagnostic purposes. However, research exploring the potential untoward effects of gold nanoparticles on female reproductive function and fertility is almost nonexistent. We have demonstrated previously that 10-nm GNPs (2.85×10^{10} particles/mL) are able to enter rat ovarian granulosa cells and modulate estrogen accumulation *in vitro* [1]. Moreover, we have shown using a rat ovarian culture model that 10-nm GNPs modulate progesterone (P₄) accumulation in a dose- and time-dependent manner, but does not alter estradiol-17 beta (E₂) accumulation. The objective of the present study was to evaluate potential time- and dose-dependent effects of GNPs (10 nm) on inhibin concentrations in culture medium by radioimmunoassay using a rat ovary culture approach. We hypothesized that gold nanoparticles would perturb ovarian inhibin secretion/accumulation in culture and ultimately diminish its production long term. Ovaries from each rat remained paired for statistical purposes; thus, one ovary was cultured under control conditions and the other under treatment conditions (2.85×10^4 particles/mL, 2.85×10^7 particles/mL, and 2.85×10^{10} particles/mL medium) for three different time periods (12, 24, and 48 hours). Two-way ANOVA results did not reveal any significant effects of incubation time or GNP concentration ($df=94$, $p>0.05$) on inhibin concentrations in culture medium relative to control. Although GNPs exert an effect on ovarian steroid secretion/accumulation *in vitro*, the present results suggest that GNPs have no effect on ovarian inhibin secretion/accumulation using our paradigm.

[1] Stelzer RV & Hutz RJ, J Reprod Develop 2009; 55:685

Sources of Research Support: NIEHS CEHSCC at UWM and CRI Pilot Proposal Program; (Grant: P30ES004184; Pilot Project: PRJ321R).

Nothing to Disclose: JKL, GW, KT, MJC, RK, RJH

Pub # P1-87

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Exposure to Low-Level Arsenic (As) Decreases Circulating Insulin-Like Growth Factor-I (IGF-I), Resulting in Delayed Female Pubertal Development

Author String JC Saca, MP Reilly, W Whitehouse-Innis, JG Parsons, RK Dearth
University of Texas Pan American, Edinburg, TX; University of Texas Health Science Center San Antonio Medical Research Division, Edinburg, TX; University of Texas Pan American, Edinburg, TX

Body Arsenic (As) is a prevalent environmental toxin due to its history of industrial use and natural leaching from the Earth's crust. The result is polluted water supplies and soil making the toxin easily accessible for human consumption. Arsenic exposure has been linked to various common human health issues including delayed female puberty in women living in areas with water supplies contaminated with high arsenic levels. Supporting this, high exposure to arsenic in adult female rats has been shown to suppress serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolong diestrus. While evidence exists to suggest that arsenic can disrupt reproductive processes responsible for pubertal development, no studies to date have shown that low-level exposure to the toxin during a critical developmental window results in delayed female puberty. Therefore, the present study investigates the ability of low-level As to disrupt key reproductive hormones and their respective impact on pubertal development. Infantile female Sprague-Dawley rats were administered a daily dose of either a 5mg/kg sodium arsenite solution (As(III)), 10mg/kg of As(III), 20mg/kg of As(III) or saline solution (control) via gastric gavage until pubertal onset. Results showed that the 5mg/kg dose had no effect on pubertal onset. However, exposure to the 10mg/kg dose resulted in a significant ($p < 0.05$) delay in vaginal opening (VO) and first diestrus compared to controls. Importantly, the 10mg/kg dose had no effect on growth compared to controls. The 20mg/kg dose also resulted in delayed VO but with a significant reduction in growth compared to controls. In a separate study, infantile female rats were exposed to 10mg/kg of As or saline until the late juvenile period (day 30). Surprisingly, chronic exposure to the 10mg/kg dose of arsenic did not alter serum levels of circulating LH, FSH, prolactin (PRL) and growth hormone (GH) compared to controls. However, arsenic significantly ($p < 0.01$) suppressed serum levels of IGF-I compared to controls. In addition, immunoblot analysis of livers exposed to arsenic showed decreased phosphorylation of ERK 1/2 compared to controls. As expected, arsenic levels were higher in brain, pituitary, liver and blood compared to controls. Overall, this is the first study to show that low-level exposure to arsenic acts peripherally (liver) to suppress circulating levels of IGF-I resulting in delayed female puberty.

Sources of Research Support: UTPA Faculty Research Council Grant awarded to RKD.

Nothing to Disclose: JCS, MPR, WW-I, JGP, RKD

Pub # P1-88

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Cadmium Acting as a Xenoestrogen Increases the Secretion of Prolactin

Author String EA Miler, FA Quinteros, BH Duvilanski
Facultad de Farmacia y Bioquímica-Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina

Body

The heavy metal cadmium (Cd^{2+}) is one of the most important environmental contaminants with many adverse effects on human health. Evidence strongly indicates that Cd^{2+} acts as an endocrine disruptor. Previous studies from our group have shown that Cd^{2+} induces an anterior pituitary cell proliferation via estrogen receptor alpha ($\text{ER}\alpha$).

The aim of this study was to investigate whether Cd^{2+} is able to reproduce another physiological response of 17β -estradiol (E_2) on anterior pituitary cells such as prolactin (PRL) release. In addition, Cd^{2+} effects on $\text{ER}\alpha$ gene expression were evaluated.

Anterior pituitary cell cultures from young-adult female rats were used. Experiments were performed in phenol red-free Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% charcoal stripped fetal bovine serum. Cells were incubated with Cd^{2+} (10 nM) or vehicle alone (control) for different times. PRL levels were measured by radioimmunoassay. PRL and $\text{ER}\alpha$ gene expression were studied by semi-quantitative PCR (GAPDH, internal control) and $\text{ER}\alpha$ protein expression by western blot. E_2 (1 nM) was used as positive control of the experiments.

Cd^{2+} stimulated PRL liberation in time dependent way (folds increase: 2 h: 0.8; 4 h: 1.5; 24 h: 3.0; 72 h: 4.4). Cd^{2+} exposure for 8 or 24 h increased PRL mRNA levels (relative expression of PRL, % of control: 8 h: Cd^{2+} : 191.6 ± 25.8 , $p < 0.05$ and 24 h: Cd^{2+} : 216.7 ± 21.9 , $p < 0.05$). Similar effects were obtained with E_2 on mRNA PRL expression (8 h: E_2 : 219.0 ± 18.9 , $p < 0.05$ and 24 h: E_2 : 297.7 ± 14.9 , $p < 0.01$). Cd^{2+} and E_2 showed a synergistic effect on mRNA PRL expression. Cd^{2+} and E_2 exposure enhanced $\text{ER}\alpha$ mRNA levels (relative expression of $\text{ER}\alpha$, % of control: 8 h: Cd^{2+} : 147.3 ± 11.8 , $p < 0.05$; E_2 : 150.6 ± 4.4 , $p < 0.05$ and 24 h: Cd^{2+} : 145.4 ± 1.4 , $p < 0.05$; E_2 : 189.4 ± 7.0 , $p < 0.01$). These increases were also manifested at the level of protein expression. On the contrary at 72 h of Cd^{2+} exposure a reduction of $\text{ER}\alpha$ mRNA levels was observed (Cd^{2+} : 60.0 ± 2.3 , $p < 0.05$). These effects were abrogated in the presence of ICI 182,780, 10^{-7} M.

The results of this study show that Cd^{2+} has a potent estrogen-like activity on anterior pituitary cells as a result of its ability to increase PRL secretion. Cd^{2+} , as E_2 , also regulates $\text{ER}\alpha$ gene expression in these cells. Cd^{2+} induced disruption of PRL and $\text{ER}\alpha$ expression could play a pivotal role in the delicate balance of neuroendocrine functions such as reproduction and development.

Nothing to Disclose: EAM, FAQ, BHD

Pub # P1-89

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Gene Expression Profiling in Rat Fetal Brain after *In Utero* Diethylhexyl Phthalate Exposure

Author String K Yuan, H Lin, Q-q Lian, R-s Ge
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Body Diethylhexyl phthalate (DEHP) is most widely utilized plasticizer and one of most ubiquitous environmental contaminants. It has been demonstrated that DEHP exposure can cause neurodegeneration in rat brain, and maternal DEHP exposure can influence the lipid metabolome in fetal rat brain. In this study, we investigated the effects of gestational DEHP exposure on gene expression profiling in rat fetal brain. Adult pregnant Long-Evans dams were treated from gestational day 12 to 21 with either 0 (control), 10, or 750 mg/kg DEHP by oral gavage. Male pups were killed at a birth and brains were removed for RNA extraction. Microarray and quantitative PCR (Q-PCR) were used to identify fetal brain gene expression levels. Using genome-wide genomic hybridization microarray analysis, we found that the expression levels of 6 genes were significantly reduced (*Prm1*, *Tcp11*, *Tnp2*, *Cnih2*, *Tuba3b* and *Yif1*) for over 2 fold in 10 mg/kg DEHP-treated brains and one gene (*Scyl1*) expression level was increased. Higher dose of DEHP (750 mg/kg) also decreased *Cyp26b1* expression levels. Interestingly, 750 mg/kg DEHP significantly increased *Pmch* expression level. The expression levels of these genes after DEHP treatment were also confirmed by Q-PCR. In conclusion, our study demonstrates that in utero DEHP exposure results in a distinct pattern of genes which are critical for brain development.

Sources of Research Support: Partially by Natural Science Funding of China (30800323).

Nothing to Disclose: KY, HL, Q-QL, R-SG

Pub # P1-90

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Do Life-History Parameters Predict Susceptibility to an Endocrine Disruptor? Thyroid Development in Anadromous and Freshwater Fish Populations

Author String EM Gray, AM Petersen, RA Bremiller, Y-O Yan, CL Buck, FA von Hippel, WA Cresko, JH Postlethwait
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Environment and Natural Resources Institute, Anchorage, AK

Body Perchlorate is a known endocrine disruptor that inhibits thyroid hormone production. Vertebrates exposed to perchlorate experience pathologies associated with thyroid disruption, particularly if exposure occurs as an organism develops, or during periods of rapid growth or metabolic change. In anadromous fishes, thyroid hormone plays an important role in preparation for migration and spawning. We recently demonstrated that perchlorate exposure during development has multiple effects on the adult phenotype of anadromous threespine stickleback (*Gasterosteus aculeatus*), including alterations in morphology, reproductive systems, and behavior. It remains unknown, however, how perchlorate exposure affects the early development and proliferation of thyroid follicles in anadromous populations, and whether this effect may be attenuated in resident freshwater populations that do not rely on thyroid hormone for migratory events. The aim of this study is to determine if patterns of thyroid follicle development vary significantly between anadromous and freshwater populations. These data will provide a baseline for the comparison of thyroid development and function in these two populations after perchlorate exposure. We imaged thyroid follicles over the course of development in lab-reared stickleback larvae descended from anadromous or from freshwater populations. Larvae from both populations were raised in identical salinity (4-6ppt) and photoperiod. Fish were fixed across a range of four to fourteen days post-fertilization (dpf), sectioned, and stained with Hemotoxylin and Eosin. The number of thyroid follicles, follicle area, follicle cell height and number, and colloid area were measured and compared between populations. We found that anadromous populations develop more thyroid follicles earlier than freshwater populations, even when raised under identical salinity conditions for five or more generations. For every metric measured, thyroid follicles in anadromous fish developed faster and larger than in freshwater fish. These data suggest that these lab-reared populations will serve as a unique opportunity to learn how perchlorate affects populations with divergent patterns of thyroid development. This study will examine how spatial, temporal, and functional development of thyroid follicles is affected by perchlorate exposure in freshwater and anadromous populations of threespine stickleback.

Sources of Research Support: National Institutes of Environmental Health Science (NIEHS)1RO1ES017039-01A1 awarded to FVH, WAC, JHP.

Nothing to Disclose: EMG, AMP, RAB, Y-LY, CLB, FAvH, WAC, JHP

Pub # P1-91

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Disruption of Thyroid Hormone Receptor-Mediated Transcription, Thyroid Hormone-Induced Purkinje Cell Dendrite Arborization and Granule Cell Neurite Extension by Polybrominated Diphenylethers

Author String T Iwasaki, K Ibhazehiebo, J Kimura-Kuroda, W Miyazaki, N Shimokawa, N Koibuchi
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Body Polybrominated diphenylethers (PBDEs) have been used as flame retardants. They are becoming ubiquitous environmental contaminant. Adverse effects in the developing brain are of great health concern. We aimed to investigate the effect of PBDE/ hydroxylated (OH)-PBDE on thyroid hormone (TH) receptor (TR)-mediated transcription, TH-induced dendrite arborization of cerebellar Purkinje cells, and neurite extension of primary cerebellar granule cells. The effect of PBDE/OH-PBDE on TR action was examined using transient transfection-based reporter gene assay. Several PBDE congeners suppressed TR-mediated transcription. Among them BDE209 showed highest suppression. TR-cofactor binding was studied by mammalian two-hybrid assay. The binding of TR β 1 with steroid receptor coactivator (SRC)-1 and Nuclear receptor corepressor (N-CoR) were not affected by BDE209. We examined TR-DNA (thyroid hormone response element, TRE) using liquid chemilumnescent DNA pull down assay and found that PBDEs that suppressed TR-mediated transcription dissociated the TR β 1 from TRE. The magnitude of suppression by several PBDEs correlated with that of TR-TRE dissociation. Chimeric receptors generated from TR and glucocorticoid receptor (GR) were used to identify functional domain of TR responsible for PBDE action. PBDEs suppressed transcription of chimeric receptors containing TR-DNA binding domain (DBD). Such suppression was not observed with chimeras containing GR-DBD. To examine the effect of PBDE on cerebellar development, we examined the change in dendrite arborization of the Purkinje cells in rat cerebellar primary culture. PBDEs that suppressed the TR-mediated transcription significantly suppressed TH-induced Purkinje cell dendrite arborization, while PBDEs that did not suppress showed no significant effect. Finally, to examine the effect of BDE209 on cerebellar granule cells, we used purified rat cerebellar granule cells in reaggregate culture. Low dose BDE209 (10^{-10} M) significantly suppressed TH-induced neurite extension of granule cell aggregate. In conclusion, several PBDE congeners may disrupt the TH system by partial dissociation of TR from TRE acting through TR-DBD and consequently disrupt normal brain development.

Nothing to Disclose: TI, KI, JK-K, WM, NS, NK

Pub # P1-92

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Exposure to Polychlorinated Biphenyls Causes Alterations in Brain Serotonin Concentrations in Swiss Webster Mice

Author String T Provost, M Peppenelli
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Body Endocrine disrupting chemicals (EDC) released into the environment cause harmful effects on wildlife and humans. Polychlorinated biphenyls (PCB) are environmental contaminants that persist in the environment and are identified EDC. The hormones melatonin and serotonin are intimately linked to many physiological processes that include the reproductive system and behavior. Therefore exposure to EDC that alter these hormones can cause deleterious effects in animals that could ultimately impact future generations. We have previously shown that serotonin n-acetyltransferase (NAT), the enzyme catalyzing the production of melatonin, and melatonin are disrupted by developmental exposure to a mixture of polychlorinated biphenyl congeners, Aroclor[reg] 1254. During further investigation we have found that serotonin is disrupted by this same PCB mixture. Adult female Swiss-Webster mice were housed under controlled conditions and fed a ground rodent diet or the same diet plus 5ppm of Aroclor[reg] 1254, PCB 136, or OH-PCB for 60 days. Food intake was determined daily and body weight was measured weekly to ensure similar nutritional intake in all groups. After 60 days of treatment brains were harvested, then homogenized to determine serotonin concentrations using a commercially available radioimmunoassay kit (MP Biomedical, Solon, OH). Serotonin concentrations were significantly depressed by exposure to Aroclor[reg] 1254 ($p < 0.01$) and OH-PCB ($p < 0.05$) but remained unchanged in PCB 136 exposed animals when compared with control animals. This alteration indicates that the decline in reproductive success in animals exposed to PCB could be caused induced by interruptions in serotonin and melatonin production. Future research will focus on the impact of PCB mixtures and hydroxylated PCB congeners on brain chemistry, reproductive success and behavior in F0, F1 and F2 generations.

Nothing to Disclose: TP, MP

Pub # P1-93

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Perinatal Exposure to Polychlorinated Biphenyls Alters Spinogenesis of Newborn Neurons in the Dentate Gyrus in Mice

Author String A-S Parent, E Washburn, A Bensen, E Naveau, J-P Bourguignon, G Westbrook
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Body Perinatal exposure to polychlorinated biphenyls (PCBs) has been associated with learning and memory deficits in animals and children (1). Although their use in lubricating oils and plastics is forbidden in USA and Europe, these environmental toxins are still detected in high concentration in animals and humans because of their high stability (2). They are known to be neurotoxic and to alter thyroid function. We hypothesized that PCBs could alter neurogenesis and synaptogenesis in the dentate gyrus (DG) of the hippocampus, a structure that is necessary for learning and memory and that is regulated by sex steroids and thyroid hormones. To study the effect of perinatal exposure to PCBs on hippocampal neurogenesis, we used transgenic mice that transiently and specifically express enhanced green fluorescent protein (EGFP) in newborn neurons in the DG (3). Dams were fed Aroclor 1254 (6 mg/kg/day), a commercial mixture of PCBs, from gestational day 6 (E6) to postnatal day 21 (P21). The exposure caused a decrease of circulating total thyroxine in P19 pups (46 vs 31 ng/ml in control vs Aroclor exposed animals), leading to a state of relative hypothyroidism. Aroclor 1254 exposure did not affect the number of newborn cells in the dentate gyrus as measured by immunohistochemistry against EGFP and confocal imaging at P7, 14 and 21. Injection of bromodeoxyuridine (BrdU) at P7 and immunohistochemistry for BrdU at P8 and P28 did not show any effect of Aroclor 1254 on the number of proliferating cells at P8 or on cell survival at P28. Nestin mRNA expression, a marker of precursor cells, in the DG of P14, 21 and 38 animals was not modified by perinatal exposure to Aroclor 1254. To examine the effects of perinatal Aroclor 1254 on dendrite and spine formation, we injected the hippocampus of P7 mice *in vivo* with a retrovirus encoding EGFP that labels newborn neurons in the dentate gyrus. At P21, dendrites of EGFP positive neurons were imaged using confocal microscopy. Perinatal exposure to Aroclor 1254 significantly increased spine density (+21%) in newborn neurons whereas it did not affect total dendrite arborization. Our results indicate that PCBs affect synaptic development in newborn neurons in the hippocampus, which could contribute to longterm effects on hippocampal functions such as learning and memory.

(1) Diamanti-Kandarakis E et al, Endo Rev 2009; 293-342

(2) Woodruff TJ et al, EHP 2010; 10.1289/EHP.1002727

(3) Overstreet LS et al, J Neurosci 2004; 3251-3259

Sources of Research Support: Fonds National de la Recherche Scientifique, Fonds Leon Fredericq (ASP) and NIH grant MH46613 (GLW).

Nothing to Disclose: A-SP, EW, AB, EN, J-PB, GW

Pub # P1-94

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Effects of Prenatal Polychlorinated Biphenyl Exposure on Female Reproductive Behavior across Two Generations

Author String BA Kermath, J Jefferson, L Thompson, FA Guarraci, AC Gore
The University of Texas at Austin, Austin, TX; The University of Texas at Austin, Austin, TX; The University of Texas at Austin, Austin, TX; Southwestern University, Georgetown, TX

Body Sex-specific reproductive behavior and physiology are crucial to the success of mammalian species. Endocrine disrupting chemical (EDC) exposure at critical perinatal developmental stages, during which the sexually-dimorphic neural circuitry that controls these behaviors is established, may cause long-term behavioral and physiological defects in adulthood. Furthermore, EDC effects may influence future generation through behavioral and/or epigenetic disruption of the fetally-exposed individuals. We are performing a two-generation study investigating the effects of perinatal EDC exposure on female adult reproductive function. Our model of prenatal exposure used an environmentally-relevant, estrogenic class of EDCs called polychlorinated biphenyls. Pregnant Sprague-Dawley rats were injected on days E16 and E18 with one of three treatments: 1 mg/kg Aroclor 1221 (A1221), a PCB mixture; 50ug/kg estradiol benzoate; or DMSO vehicle. Female offspring were tested for reproductive behavior in adulthood in non-paced mating trials with sexually-experienced, untreated males. Receptive, proceptive, aggressive and defensive female behaviors were scored for the first 10 male copulatory acts. After behavioral testing, females carried litters to term, and the F2 offspring were also observed for reproductive behavior in adulthood. For both generations, rats were euthanized, and hypothalamic regions were frozen for future qPCR, and serum banked for hormone RIAs. Currently we have preliminary behavioral data for the F1 female offspring (n = 5-7/group). Perinatal EDC exposure did not affect lordosis quotient or rating of lordosis intensity, compared to DMSO. However, rats exposed to EB and A1221 treatment took longer to display the first lordotic response to male approaches when compared to control. Proceptive, aggressive and defensive female behaviors are currently being scored from the videotaped trials. Our preliminary data indicate that prenatal EDC exposure disrupts female reproductive behavior, specifically by delaying the expression of receptive behavior during a mating encounter. These data have implications for wildlife and human reproductive function, as neuroendocrine pathways controlling reproduction are highly conserved across species. Ongoing studies will extend the current work to transgenerational effects in the F2 generation.

Sources of Research Support: NIH IRC1 ES018139.

Nothing to Disclose: BAK, JJ, LT, FAG, ACG

Pub # P1-95

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Prenatal Polychlorinated Biphenyls (PCBs) Alter Male-Typical Sexual Behavior in the Rat

Author String J Jefferson, BA Kermath, L Thompson, FA Guarraci, J Dominguez, AC Gore
The University of Texas at Austin, Austin, TX; The University of Texas at Austin, Austin, TX; The University of Texas at Austin, Austin, TX; The University of Texas at Austin, Austin, TX; Southwestern University, Georgetown, TX

Body Polychlorinated biphenyls (PCBs) are a class of endocrine disrupting chemicals (EDCs) that persist and bioaccumulate in the environment. Perinatal exposure to PCBs during critical developmental stages disrupts the sexual differentiation of the brain, potentially altering the activation of sexual behaviors in adulthood. To test the effects of prenatal PCB exposure on adult masculine reproductive behavior we injected pregnant rats with one of three treatment groups at gestational days 16 and 18: 1mg/kg of Aroclor 1221 (A1221, an estrogenic PCB mixture), 50[μg/kg of estradiol benzoate (EB) or the vehicle dimethyl sulfoxide (DMSO). The offspring were allowed to mature, and tested as young adults (P60) for male-typical mating behaviors [mount frequency (MF), intromission frequency (IF), latencies to mount, intromit, and ejaculate, and the postejaculatory interval]. Mating trials were videotaped and scored by observers who were blind to the treatment condition of the subjects. Males were later euthanized to collect serum for measuring circulating hormone levels, and their testes and adrenals were weighed. Brain dissections were taken from the preoptic area and mediobasal hypothalamus for future qPCR analysis. Our preliminary behavioral data (n = 5-7) show a decreased latency of the prenatally PCB exposed males to mount and intromit, decreased postejaculatory interval, but an increase in the latency to ejaculate, compared to DMSO-treated males. Estradiol-exposed males only showed an increase in the latency to ejaculate compared to DMSO-treated males. Thus, our preliminary data suggest that prenatal low-dose PCB exposure has discrete effects on appetitive and consummatory male sex behaviors, and that A1221 may also be acting via non-estrogenic mechanisms as its effects differed from EB. We are currently performing similar reproductive behavioral tests of the F2 generation to look at transgenerational impacts of EDCs. The results of these studies will have implications for neuroendocrine disruption by PCBs in wildlife and humans, as the neuroendocrine control of sexual behavior is highly conserved.

Sources of Research Support: NIH 1RC1 ES018139.

Nothing to Disclose: JJ, BAK, LT, FAG, JD, ACG

Pub # P1-96

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Prenatal Endocrine Disruption Affects Endocrine Systems across the Life Cycle

Author String DM Walker, NQ Dinh, S Tillekeratne, J Yoon, AC Gore
The University of Texas at Austin, Austin, TX; The University of Texas at Austin, Austin, TX; The University of Texas at Austin, Austin, TX

Body Polychlorinated Biphenyls (PCBs) are industrial contaminants and a class of known endocrine disrupting chemicals. Exposure to PCBs during perinatal development results in long-term alterations in numerous reproductive endpoints in rodents such as advancing or delaying the timing of puberty and reproductive senescence. However, little is known regarding the process by which these long-term alterations take place. We tested the hypothesis that early life exposure to Aroclor 1221 (A1221), a commercially available mixture of lightly chlorinated PCBs, results in long-term alterations to reproductive parameters that are closely associated with changes to endocrine tissues (pituitary, adrenals and gonads) throughout the body as well as gene expression changes in specific nuclei in the hypothalamus known for regulating reproductive function. Methods: Pregnant Sprague Dawley rats were injected on embryonic day 16 and 18 with vehicle (DMSO; N=22), A1221 (1mg/kg; N=23) or estradiol benzoate (EB, 50 [micro]g/kg; N=24). Dams were allowed to give birth and developmental milestones such as eye opening, the timing of puberty, estrous cyclicity and timing of reproductive senescence were monitored in the offspring throughout their life cycle, from birth through aging. On postnatal days (P) 15, 30, 45, 90 and ~270, 1 male and 1 female from each litter was euthanized. Brains and serum were frozen for RNA extraction and radioimmunoassays respectively. Pituitaries, adrenals and gonads were dissected and weighed to determine effects of EDC exposure on the endocrine tissues throughout development. Results: Significant sex and age effects were observed on body weight, gonadal/somatic index, adrenal weight and pituitary weight. The adrenals were larger in males and females treated with EB and A1221 at several ages. Finally, in the aging animals (~P270) body weights and pituitary and adrenal weights were affected by treatment and housing social condition (larger in EB, smaller in A1221), an effect that was greater in males. Conclusions: These data suggest that gestational exposure to EB and A1221 have age and treatment specific effects on endocrine and somatic tissues. These are the first steps in our ongoing study; next, we will utilize PCR-based arrays to assess expression of hypothalamic gene expression, and to measure hormones in animals exposed to perinatal endocrine-disrupting chemicals.

Sources of Research Support: NIH F31 AG034813-01A1 - DMW; NIH RC1 ES018139 - ACG.

Nothing to Disclose: DMW, NQD, ST, JY, ACG

Pub # P1-97
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)
Title Disruption of Cell Cycle Exit and Neuronal Migration after Fetal Rat Exposure to Polychlorinated Biphenyls
Author String A-S Parent, E Naveau, A Gerard, J-p Bourguignon
University of Li[grave]ge, Li[grave]ge, Belgium

Body Polychlorinated biphenyls (PCBs) are endocrine disrupting chemicals (EDCs) present in lubricating oils and plasticizers. Though they have been banned, a very recent NHANES study reported detectable PCBs in 99% of American pregnant women (1). Perinatal exposure to PCBs has been reported to cause moderate cognitive deficits in children. However, the underlying cellular and molecular mechanisms are only partially understood. Beside their neurotoxic effects, PCBs alter the action of thyroid hormones which play fundamental role in the development of the cortical brain (2). The first aim was to study the effects of PCBs on cell death in astrocytes and neurons primary cultures in vitro. Secondly, we studied the effects of perinatal exposure to PCBs on neuronal progenitor proliferation, survival and migration in the cerebral cortex. Using astrocytes and astro-neuronal primary cultures, cell death was studied through TUNEL labeling after incubation during 4h, 8h or 24h with different concentrations (1.5 to 100 [micro]M) of Aroclor 1254, a commercial mixture of PCBs. After 8h of exposure, the highest Aroclor concentrations induced cell death in astro-neuronal cultures and this effect became significant after a 24h exposure. Such an effect was not seen in pure astrocytes cultures. After prenatal exposure to Aroclor 1254 (6 mg/kg/day orally in dams from E6 to P0), the cerebral cortex was studied through DAPI, KI67 and BrdU immunocytochemistry. The PCBs did not affect neither neuronal progenitor proliferation nor cell death in the subventricular zone of the cerebral cortex of rat embryos at E17, E19 and P0. At E17, the cell cycle exit was increased in the subventricular zone of rat pups exposed to Aroclor 1254 in comparison with controls. At E20, radial neuronal migration was delayed in the caudal cortex after developmental exposure to Aroclor but the number of Sox5 positive neurons in the inner cortical layers was not modified. In conclusion, developmental exposure to Aroclor 1254 alters cell cycle exit and radial neuronal migration in specific areas and in a critical age window but does not affect progenitor proliferation. These region specific effects could be due to the cortical distribution of the aryl hydrocarbon receptor and the rostro-caudal progression of cortex development.

- (1) Woodruff TJ et al, EHP 2010; 10.1289/EHP.1002727
(2) Diamanti-Kandarakis E et al, Endo Rev 2009; 293-342

Sources of Research Support: Fonds National de la Recherche Scientifique (FNRS Belgium).

Nothing to Disclose: A-SP, EN, AG, J-PB

Pub # P1-98

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Perchlorate-Sensitive NIS Symporter and Its Paralogs Are Expressed in Developing Thyroid, Gonad, and Neural Crest Cell-Derived Tissues in the Threespine Stickleback (*Gasterosteus aculeatus*)

Author String AM Petersen, Y-L Yan, RA Bremiller, EM Gray, CL Buck, FA von Hippel, JH Postlethwait, WA Cresko
Center for Ecology and Evolutionary Biology, Eugene, OR; Institute of Neuroscience, Eugene, OR;
Environment and Natural Resources Institute, Anchorage, AK

Body Perchlorate is a pervasive contaminant in the U.S. and a known disruptor of thyroid hormone production in humans. Perchlorate competitively inhibits the sodium-iodide symporter (NIS, alias SLC5a5), blocking iodide availability for thyroxin production, leading to hypothyroid condition in exposed humans and animals. SLC5a5 has several paralogs with important and varied function in vertebrate tissues. We have recently demonstrated that perchlorate affects gonad development in a vertebrate model organism, the threespine stickleback. We hypothesize that reproductive development may be affected by perchlorate through disruption of function of SLC5a5 paralogs in non-thyroidal tissues. We cloned the *slc5a5* gene and its paralogs *slc5a6a*, *slc5a6b*, and *slc5a8*. We employed in situ hybridization to determine the spatial and temporal distribution of SLC5a5 transcript during early development, 4-14 days post fertilization (dpf) and immunohistochemistry to assess Thyroglobulin (Tg) protein expression in the same period. Results showed that at 8 dpf, *slc5a5* was expressed in ethmoid, swim bladder, and somites and at 14 dpf, in skeleton and teeth. This expression pattern suggests a role for SLC5A5 in developing neural crest derivatives. Beginning at 11 dpf, *slc5a5* and Tg staining were co-localized, showing that *slc5a5* is expressed in thyroid tissue in stickleback. We found widespread expression of *slc5a6a*, *slc5a6b*, and *slc5a8* after 11 dpf, with *slc5a6a* and *slc5a6b* transcript distributed throughout the branchial region, in eye, somites and gut and *slc5a8* in connective tissue, cartilage, teeth, and in the thyroid. All three paralogs were expressed in or near thyroid tissue at 14 dpf. This pattern was similar in 11 dpf fish, but not visible at 8 dpf. In 9 month-old fish, we identified expression of these paralogs in the gonad of males and females. These data suggest that the development of thyroid tissue and the initiation of expression of NIS symporter paralogs occur between 8 and 11 dpf in normally developing stickleback. We demonstrated that *slc5a5* and its paralogs are also expressed in the gonads and neural crest cell-derived tissues. These data suggest that perchlorate could profoundly affect multiple tissues during development via disruption of SLC-family symporter function in non-thyroidal tissue. We are currently testing this hypothesis.

Sources of Research Support: National Institute of Environmental Health Sciences(NIEHS)RO1 Grant ES017039-01A1 awarded to FVH, JHP, WAC.

Nothing to Disclose: AMP, Y-LY, RAB, EMG, CLB, FAvH, JHP, WAC

Pub # P1-99

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Alternative Splicing of Genes Encoding Estrogen Receptor α in Zebrafish (*Danio rerio*) and Killifish (*Fundulus heteroclitus*): Regulation by Tissue Type, Sex, Stage of Development, and Chemical Exposure

Author String KA Cotter, A Yershov, DE Nacci, GV Callard
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Body The diversity and complexity of estrogen's regulatory functions are mediated by two or three genetically distinct estrogen receptors (ER) and multiple structurally and functionally different splice variants. Although it is well-established that estrogen-like environmental chemicals interact with the ligand binding site of ER to disrupt transcriptional control mechanisms, the possibility that these chemicals also impact splicing decisions on estrogen responsive genes, such as that encoding ER α , has been largely unexplored. A targeted PCR cloning approach was used to survey ER α mRNA variants in zebrafish, a convenient laboratory model, and killifish, an environmentally relevant species. At least six mRNA variants were identified in zebrafish and eleven in killifish, several of which were common to both species. Sequence analysis revealed use of multiple transcription/translation start sites, exon deletions, intron sequence retention /premature termination, and alternative 3'-UTRs and poly-A sites. N-terminal variants predicting 'long' (L) and 'short' (S) ER α isoforms were selected for detailed analysis. As measured by quantitative (Q)PCR, total ER α mRNA and S:L ratio differed by tissue type, sex, stage of development and chemical exposure. For example, in zebrafish brain, eye and heart, the transcript encoding the long isoform was significantly more abundant than the short isoform (S:L, >1:100) and accounted for the majority of total ER α mRNA. A much higher proportion of short variant was found in testis (1:50), ovary (1:10), and liver (1:2). Dosing with estradiol and estrogen-like chemicals upregulated total ER α in zebrafish embryos by selectively upregulating the short variant >150-fold. Results were similar with killifish embryos. Significantly, embryos derived from a killifish population exposed to estrogenic contaminants in a polluted (New Bedford Harbor, NBH), as compared to an unpolluted (Scorton Creek, SC), environment had 5-fold higher levels of total ER α mRNA due mainly to high expression of the short isoform. Although further analyses are required to determine if the S:L ratio or one or more other ER α variants are somehow related to endocrine disruption and estrogen hyporesponsiveness observed in NBH killifish (1), results support the idea that environmental estrogens exert at least some of their effects by altering the nature or expressed levels of structurally and functionally distinct ER α isoforms.

(1) Greytak et al., *Aquat Toxicocol* 99:291, 2010

Sources of Research Support: Boston University Superfund Research Program (P42 ES07381).

Nothing to Disclose: KAC, AY, DEN, GVC

Pub # P1-100

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Synergistic Effects of Combination of Octylphenol and Isobutyl Paraben on the Induction of *Calbindin-D9k* Gene in GH3 Cells

Author String Y-R Kim, E-B Jeung
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Body Endocrine disruptors (EDs) have estrogenic activities and cause physiological estrogenic responses. Octylphenol (OP) is one of alkylphenilic compounds that are called as environmental estrogens because they strongly compete with endogenous estrogens for binding to the estrogen receptor (ER). Calbindin-D9k (CaBP-9k) has been shown to be a novel biomarker for detecting EDs. In this study, CaBP-9k gene was employed to examine the estrogenic activity of mixture of parabens and OP for an additive, a synergistic, or an antagonistic effect in rat pituitary GH3 cells. GH3 cells were treated with OP, isobutyl paraben (IBP) or their combination in a dose-dependent manner (single: 10^{-6} , 10^{-7} , and 10^{-8} M, combination: OP 10^{-6} to 10^{-8} + IBP 10^{-6} to 10^{-8} M). To determine the time dependency, the cells harvested at different time points (6, 12, 24, and 48 h). ICI 182,780, was used to examine the potential involvement of ER in the induction of CaBP-9k by EDs via an ER-mediated pathway. Treatment with combination of OP and IBP induced significant increase in CaBP-9k expression in a dose-dependent manner. CaBP-9k mRNA and protein expression peaked at 24 h following treatment. Co-treatment with ICI 182,780 significantly attenuated ED-induced CaBP-9k expression in GH3 cells. Taken together, these results indicate that combined exposure of the cells to OP and IBP may have a synergic effect on the induction of CaBP-9k gene in GH3 cells.

Nothing to Disclose: Y-RK, E-BJ

Pub # P1-101

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Synergistic Effects of Paraben Combinations on the Induction of *Calbindin-D9k* Gene Via a Progesterone-Receptor Mediated Pathway in GH3 Cells

Author String H Yang, K-C Choi, E-B Jeung
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Body Although an endocrine disrupting bioactivity of parabens, xenoestrogens, has been known to have weak estrogenic activities, synergic estrogenic effect(s) of these paraben combinations remains to be elucidated. The purpose of this study was to investigate the synergic effects of an individual paraben including methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP), isopropyl paraben (IPP), butyl paraben (BP), and isobutyl paraben (IBP) and combinations of parabens (MP+EP+PP+BP; PP+IPP; and BP+IBP) in GH3 cells on the induction of an estrogenic biomarker, *calbindin-D9k* (*CaBP-9k*) gene. *CaBP-9k* mRNA and protein expressions were analyzed by real-time PCR and Western blot analysis, respectively. Following 24 h treatment, a significant increase in *CaBP-9k* expression was observed depending on the linear length of the alkyl chains from MP to IBP. Interestingly, the synergistic effect of an actual concentration of paraben combinations was observed at a low dose (10^{-5} M), which induced the highest expressions of *CaBP-9k* mRNA and protein. To investigate the involvement of estrogen receptors (ERs) and progesterone receptors (PRs) in which paraben combinations exert their effects, the expressions of *ERa* and *PR-B* were simultaneously examined. The mRNA and protein expressions of *ERa* fluctuated. In addition, *ERa* was not shown the inhibition when parabens co-treated with ICI 182,780, indicating that *CaBP-9k* may not be induced through an ERa-dependent pathway. The paraben combinations induced *PR-B* gene expression and its expression was totally abolished by ICI 182,780, implying that *CaBP-9k* expression may be involved with a PR-mediated estrogenic activity of paraben combinations in GH3 cells. Taken together, these results suggest that exposure to the low concentration of paraben combinations may increase the synergic estrogenic activities in GH3 cells through a PR-involved pathway.

Nothing to Disclose: HY, K-CC, E-BJ

Pub # P1-102

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Exposure to Parabens Disrupts Ovarian Follicle Maturation in a Rat Model

Author String H-J Ahn, E-B Jeung
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Body Parabens are widely used as an antimicrobial agent in the cosmetic consumer products and pharmaceutical preparation industries. Parabens are esters of *para*-hydroxybenzoic acid typically including methyl-, ethyl-, buthyl-, isobutyl-, and isopropylparaben. Recently, parabens have been shown to act as xenoestrogens, a class of endocrine disruptors. Several studies have reported that exposure to estrogenic chemicals changed normal reproductive tissue development in the uterus during lactation period. In this study, we hypothesized that treatment with parabens may disrupt ovarian follicle maturation in female Sprague-Dawley rats during the juvenile-peripubertal period. The rats were orally treated with these parabens at postnatal day 21-40 in a dose-dependent manner (62.5, 250 and 1,000 mg/kg body weight (BW)/day). 17 α -ethinylestradiol (1 mg/kg BW/day) was used as a positive control and corn oil as a negative control. The ovaries were excised from the peripubertal rats and then these tissues were fixed and stained with hematoxylin and eosin for histological analysis. The number of follicles counted and measured, and divided into the preantral and antral follicles in the definite area of the ovary. In this study the number of preantral follicles in the ovaries significantly increased by propyl and Isopropyl parabens when compared to a vehicle. Almost ovarian follicles undergo an atretic degradation process such as apoptotic cell death during ovarian follicular development. Thus, we examined follicle apoptosis in granulosa cells by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling). Atretic follicles were induced by propyl and Iso-propyl parabens when compared to a vehicle. Taken together, these results indicate that two parabens (propyl and iso-propyl) may have an estrogenic effect to disrupt ovarian follicle development among other parabens on reproductive function in a rat model.

Nothing to Disclose: H-JA, E-BJ

Pub # P1-103

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Triclosan Exposure Enhances the Uterotrophic Response to Low Doses of Ethinyl Estradiol (EE) in the Female Wistar Rat

Author String GW Louis, D Best, TE Stoker
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Body Triclosan [TCS; (5-chloro-2-(2, 4-dichlorophenoxy) phenol)] is an antimicrobial agent commonly found in personal care and sanitizing products, such as soaps, toothpaste and hair products. Recent studies have identified TCS in human breast milk, blood and urine. TCS has also been reported to alter endocrine function by decreasing thyroxine and testosterone in rats; thus, raising new concerns that this antimicrobial may adversely affect human health. Previously, we reported that oral exposure to TCS (150 mg/kg) advanced the age of vaginal opening and increased uterine weight, indicative of an estrogenic effect. In the weanling rat, TCS alone had no effect on uterine weight, whereas a combined exposure to EE (3 [micro]g/kg) and TCS (4.65 mg/kg and greater) potentiated the effect of EE on uterine growth (i.e., uterine weight and epithelial cell height). To further characterize the combined action of TCS and EE, we examined uterine growth in the immature rat following EE treatment at concentrations that are more representative of pharmaceutical doses (0.125 to 3 [micro]g/kg). EE at concentrations as low as 0.25 [micro]g/kg significantly increased uterine weight compared to vehicle alone, whereas EE at 0.125 [micro]g/kg had no effect. We found that 9.375 and 18.75 mg/kg of TCS in combination with EE (0.25 [micro]g/kg and higher) resulted in approximately a 25% increase in uterine weight compared to the uterine weights of those exposed to EE alone. In addition, the lowest dose of TCS tested (4.69 mg/kg) increased uterine weight greater than that observed in the weanlings exposed to EE alone only at the higher doses of EE (2 and 3 [micro]g/kg). Thus, TCS enhances the uterine response to concentrations of EE that are lower than previously shown. The significance of these observations lies in the fact that the potentiation of EE by TCS was observed at doses of EE comparable to those used for birth control and post-menopausal management. Because the doses of TCS used were greater than exposures in humans, the implications for human health remain to be determined. The mechanism(s) through which TCS enhances EE activity is under examination. *This abstract does not necessarily reflect EPA policy.*

Sources of Research Support: NCSU/EPA Cooperative Training Program in Environmental Sciences Research, Training Agreement CT833235-01-0 with NC State University.

Nothing to Disclose: GWL, DB, TES

Pub # P1-104

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title The Effect of Chronic Exposure to Carbendazyme on the Gonads and Reproduction of Rats

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Medical University of Silesia, Katowice, Poland; Hospital of the Ministry of Interior and Administration,
Katowice, Poland

Body

Introduction: The carbendazyme is applied in the cultivation of plants as a fungicide. It caused morphological and functional changes in the reproductive system and had a teratogenic effect among experimental animals. However, the results of the research were not clear.

Aim: The aim of study was to examine the morphology and function of gonads and to check reproduction index among rats after 24 months' use of carbendazyme.

Material and methods: To determine two generations test reproduction index, 448 animals were used. The examinations of gonads morphology and serum hormones concentrations: estradiol-E2, free testosterone-FT and LH were led among 48 rats. The animals were divided into four groups. One was not exposed to carbendazyme. The other three received food with carbendazyme in growing dosages: first -5 mg/kg body weight (bw)/day, second -25 mg/kg bw/d and third-125 mg/kg bw/d. every day for two years. In two generations test the influence of the compound on reproductive system was evaluated: estrous cycle, behaviour at mating time, fertilization, delivery, feeding, taking away, growing and developing of the offsprings. The sperm was taken from all the males of the first generation (P) and of the second one (F1) during the section. The amount of sperm cells and the percentage of correct forms was established. Pathomorphological exams of pituitary glands and gonads were performed among the generation P after the 24-months. The concentrations of: E2, FT and LH in serum were also evaluated.

Results: The negative influence of the compound on fertility was not demonstrated in the two generations test. The observation of the newborns of F1 and F2 generation did not reveal any changes in their bodies. Besides, any pathological changes were observed neither in dead animals nor stillborns. The index determining sperm was comparable among all examined groups and in the controls for generations P and F1. No effect of the compound on levels of E2, FT and LH as well as gonads' morphology were observed. The pathomorphological changes in pituitary glands were more frequent in males than in females, however they were independent from applied dosage of the compound.

Conclusions: 1. Chronic application of carbendazyme in rats, regardless of their sex doesn't cause any pathomorphological changes in gonads and concentrations of sex hormones, 2. Carbendazyme applied at different dosage in rats doesn't influence the reproduction index, as examined in two generations test.

Nothing to Disclose: CM, TS, KK, BU-L, TJI, MM-O

Pub # P1-105

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Magnesium Deficiency in Pregnant Rats Alters Methylation of Specific Cytosines in the Hepatic Hydroxysteroid Dehydrogenase-2 Promoter of the Offspring

Author String J Takaya, A Iharada, Y Okihana, K Kaneko
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Body Background: Prenatal under-nutrition involves changes in the epigenetic regulation of specific genes. Epigenetic regulation of genes, specifically methylation of clusters of cytosine-guanine (CpG) dinucleotides and covalent modifications of histones in promoter regions, are established early in life and confer stable silencing of transcription. Maternal magnesium (Mg) deficiency affects maternal glucocorticoid metabolism, but the mechanisms underlying changes in glucocorticoid homeostasis of offspring are not well understood. In this study, we investigated the effects of feeding pregnant rats a Mg-deficient diet on the methylation of CpG dinucleotides in hepatic glucocorticoid genes of neonatal offspring, compared with the controls. Methods: Female Wistar rats consumed Mg-deficient (0.003% magnesium) or control (0.082% magnesium) diets ad libitum from 3 wk preconception to 21 d postparturition. Litters were culled to 8 pups/dam postnatally and pups were allowed to nurse their original mothers. Offspring were killed on day 21. Methylation of CpG dinucleotides in the peroxisome proliferator-activated receptor α (PPAR α), glucocorticoid receptor (GR) and 11 β -hydroxysteroid dehydrogenase-2 (11 β -HSD2) promoters in the liver were measured by pyrosequencing. Quantitative real-time PCR was used to assess hepatic mRNA expression of each gene. Results: Mean methylation of the 11 β -HSD2 promoter in the Mg-deficient offspring (33.2%) was higher than in controls (10.4%). This was due to a specific increase at CpG dinucleotides 1 (20.0% vs control 10.1%), 2 (58.8% vs 17.0%), 3 (29.7% vs 6.2%), and 4 (38.7% vs 8.8%) (P<0.05). PPAR α and GR methylation status and expression did not differ between the groups. No significant difference was noted between male and female pups, which were equally represented. Conclusions: Our results show that prenatal Mg deficient induces persistent, gene-specific epigenetic changes that alter mRNA expression. Although an association with methylation and mRNA expression of 11 β -HSD2 was not seen, possible relationship with other epigenetic components of 11 β -HSD2 remain to be determined. A Mg-deficient diet alters glucocorticoid metabolism, predicting higher hepatic intracellular glucocorticoid concentrations, and is possibly a key mechanism that induces the metabolic complications of Mg deficiency.

Nothing to Disclose: JT, AI, YO, KK

Pub # P1-106

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title *In Utero* Exposure to Perfluorooctane Sulfonate Lowers Fetal Body Weight in Rats

Author String Y Zhang, B Zhao, Y Chu, K Yuan, Q-q Lian, R-S Ge
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Body Excessive glucocorticoid exposure to fetus has been shown to lower birth weight of neonates. The entry of maternal glucocorticoid (corticosterone in rats) into fetal circulation is controlled mainly by 11 β -hydroxysteroid dehydrogenase 2 (11 β HSD2). Perfluorooctane sulfonate (PFOS), a widely used chemical, has been shown to have endocrine disrupting action, including potent inhibition of 11 β HSD2 activity. Thus, the suppression of placental 11 β HSD2 may impact on fetal growth. The present study investigated the effects of in utero exposure to PFOS on fetal growth and placental function. Pregnant Sprague-Dawley dams were treated with 0, 5 and 20 mg/kg body weight PFOS from gestational day 12 to 18. Placental weight, fetal body weight and length as well as placental specific genes were examined at gestational day 18. 11 β HSD2 activities in placenta were measured. 20 mg/kg PFOS significantly reduced placental weight (442.8 ± 7.4 mg, mean \pm SEM) compared to control (480.4 ± 9.7 mg). It also significantly decreased fetal body weight (2.47 ± 0.07 g) compared to control (2.77 ± 0.03 g) as well as fetal body length (2.71 ± 0.03) compare to control (3.2 ± 0.03), while 5 mg/kg PFOS did not. Exposure to higher dose of PFOS (20 mg/kg) also downregulated the expression levels of genes specific for placental growth including peroxisome proliferator-activated receptor α (*Ppara*) and γ (*Pparg*), vascular endothelial growth factor (*Vegf*), glucose transporter type 3 (*Glut3*) and 11 β HSD2 (*Hsd11b2*). PFOS (20 mg/kg) also decreased 11 β HSD2 protein levels and activity. In contrast, maternal serum corticosterone was significantly increased after PFOS treatment. Our data suggest that restriction of fetal growth in PFOS-treated rats is mediated partially via altered placental function after increasing placental glucocorticoid exposure.

Sources of Research Support: National Natural Science Foundation of China (81070329).

Nothing to Disclose: YZ, BZ, YC, KY, Q-QL, R-SG

Pub # P1-107

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title A Novel Sensitive and Selective Real-Time Cellular Assay for Detection of Endocrine Disruptors Using Native Endocrine Signaling Pathways

Author String C Jin, Y Abassi, X Xu, X Wang
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Body A cell based assay for detection of endocrine disruptors was developed using real time impedance monitoring of native endocrine signaling pathways in T-47D breast cancer cell line. T47D cells express estrogen receptor (ER) as well as some other steroid hormone receptors such as progesterone receptor (PR) and glucocorticoid receptors (GR). Stimulation of T47D cells with respective endocrine agonists leads to an increase in cell number which can be detected by gold microelectrodes embedded in the bottom of the well of specialized microelectronic plates. Addition of estrogen agonists including 17beta-estradiol, DPN and PPT to T47D cells induce a dose-dependent increase in impedance readout. The observed EC₅₀ values are better than existing *in vitro* cell based reporter assay systems. The specificity of the assay for ER activity was established using well characterized ER antagonists, such as ICI 182780. Using this assay system, several environmental hazardous compounds known to disrupt endocrine signaling pathways were tested, including bisphenol A, nonylphenol, and PCB-126. All of these compounds induced a dose-dependent impedance-based response profile similar to ER agonists and were inhibited by ER antagonists. The calculated EC₅₀ values were comparable to lowest published EC₅₀ values generated in different assay systems. Moreover, the T-47D system was also used to detect response to PR agonist, progesterone and GR agonist, dexamethasone. These two compounds also produced impedance-based biphasic responses although with different kinetic profile compared to ER activation. The data suggests the T47D assay system has the capacity to sensitively, selectively and quantitatively detect three different nuclear hormone responses.

Disclosures: CJ: Employee, ACEA Biosciences. YA: Employee, ACEA Biosciences. XX: Chairman, ACEA Biosciences. XW: Chief Scientific Officer, ACEA Biosciences.

Pub # P1-108

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Development of a Radioimmunoassay for the Measurement of Urinary Bisphenol A-Glucuronide (BPA-G)

Author String C Harthe, D Achaintre, S Rinaldi, M Pugeat, H Dechaud
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Body To overcome the dilemma of potential contamination of BPA released by the material used for urine collection or sample handling during laboratory analyses, we have developed a radioimmunoassay for the measurement of the conjugated form of BPA (BPA-glucuronide, BPA-G). This form of BPA is produced by the liver, and therefore represents the BPA ingested by the subject.

Methods: We have used BPA labelled with iodine 125 and polyclonal anti-BPA antibody (1) and a standard BPA-G kindly provided by Dr. Hidetomo Iwano (Japan). The polyclonal antibodies had 95% cross-reactivity with BPA-G, in agreement with the high specificity of our BPA-antibody to the central part of the BPA molecule. Unconjugated BPA was eliminated by a preliminary extraction step with dichloromethane (DCM).

Results: Dilution tests had a linear profile ($y = 0.86x - 0.39$; $r^2 = 0.85$) and mean recovery after loading tests was 94%. The intra- and inter-assay coefficients of variation were 13 and 14% respectively at a BPA-G concentration of 1 [$\mu\text{g/L}$], and 6% at a BPA-G concentration of 4 [$\mu\text{g/L}$]. The detection limit was 0.16 [$\mu\text{g/L}$]. On urine samples, loaded with high amounts of BPA, solvent extraction eliminates more than 90% of unconjugated BPA. The comparison of total BPA measurements on 12 urine samples obtained by RIA and a method developed by gas chromatography/mass-spectrometry (GC-MS) considered as reference method, gave very high correlation coefficients ($y = 1.03x + 1.19$, $r[\text{sup}2] = 0.95$). Specific comparisons of BPA-G concentrations obtained by radioimmunoassay and GC-MS are ongoing. The mean (\pm SD) BPA-G concentrations measured on a morning spot urine sample from 24 healthy subjects was 6.5 ± 4.2 [$\mu\text{g/L}$], ranging from 2.6 to 18.4 [$\mu\text{g/L}$]. These preliminary results are consistent with those described in the literature.

Conclusion: This assay is a suitable method to accurately measure BPA-G in DCM treated urine samples, and could be a precious tool to assess risks related to BPA exposure in populations from large clinical trials or epidemiological studies.

(1) Kaddar N et al. Anal Chim Acta. 2009; 645:1-4

Nothing to Disclose: CH, DA, SR, MP, HD

Pub # P1-109

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Sulfation of Bisphenol A by Recombinant Human Liver SULT1E1 (Estrogen Sulfotransferase)

Author String RJ Anderson
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Body Bisphenol A (BPA), a xenoestrogen found in water, polycarbonate-lined containers and dental materials, is an endocrine-disrupting chemical with potential adverse effects on normal function of human hormone-sensitive tissues. Metabolism of such xenoestrogens via sulfation, an important pathway for the metabolism of phenolic chemicals, may alter the responses to these compounds. Sulfotransferases (SULTs) catalyze the reaction using 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as the sulfuryl donor. Multiple SULTs including SULT1A1 use BPA as a substrate. We tested the hypothesis that recombinant human liver SULT1E1 (estrogen sulfotransferase) would metabolize BPA by using a radiochemical enzymatic assay with cDNA-encoded SULT1E1 expressed in COS-1 cells. SULT1E1 activity was detected with BPA as the substrate, and several biochemical properties were characterized. With varying concentrations of BPA, the activity was biphasic. Apparent Km values with BPA were 0.125 [micro]M and 12.2 [micro]M at peak BPA concentrations of 1 and 133 [micro]M, respectively. When assayed with 100 [micro]M BPA, the apparent Km with ³⁵S-PAPS was 0.24 [micro]M, the 50% inactivation temperature was 42.9[deg]C, and the IC₅₀ value with the inhibitor 2,6-dichloro-4-nitrophenol was 2.3 x 10⁻⁵ M. Activity was enhanced 2.36-fold in the presence of 100 mM NaCl.

Conclusion: Sulfation of BPA by SULT1E1 may play an important role in the overall metabolism and response to this endocrine-disrupting chemical.

Sources of Research Support: Veterans Affairs Medical Research Service.

Nothing to Disclose: RJA

Pub # P1-110

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title The Effects of Oral Bisphenol A Exposure in the Developing CD-1 (Swiss) Mouse

Author String EL Kendig, CB Lo, CJ Cookman, SM Christie, DR Buesing, R Gear, J Kendziorski, K Ungi, T McCutchan, SM Belcher
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Body The endocrine disrupting effects of Bisphenol A (BPA) have been scrutinized in recent years. In part, this is because of its wide-spread use and high volume of production worldwide, making BPA a high-value target for regulators and a seemingly irreplaceable commodity for producers. While, numerous studies have described the endocrine-disrupting effects of BPA, these studies have been criticized for not following regulatory guidelines of good laboratory practice (GLP). Those studies that have followed GLP have found few effects at human exposure levels. Many of these studies have lacked the necessary sensitivity due to wide-spread and variable background contamination with BPA and other endocrine disrupting chemicals (EDCs) that fall within the range of human exposure, making any control over exposure impossible. To address the limitations of sensitivity, background contamination has been reduced to the extent that it is possible in the current study by use of polyethylene caging and water bottles, UV-treated and charcoal-filtered drinking water, sterile woodchip cage bedding, and highly purified, reduced phytoestrogen, chow. By reducing background contamination within the experimental system, it becomes clear that BPA does alter sexual development and function in the CD-1 mouse. Similarly, BPA can affect other systems known to be sensitive to the endogenous hormone, 17 β -estradiol, but not normally considered endocrine responsive systems, specifically, cardiac function. Reducing background contamination clarifies effects in timing of puberty onset and initiation of estrous cycle as well as physical characteristics such as anogenital distance; however, more surprising is BPA's ability to alter cardiac rhythm and response to stress. Reduced background contamination affords greater sensitivity to detect EDC effects in the human-relevant dose range and will be important in determining the true nature of these chemicals in mammalian systems.

Sources of Research Support: NIH T32 ES016646-02, and NIEHS RC2 ES018765.

Nothing to Disclose: ELK, CBL, CJC, SMC, DRB, RG, JK, KU, TM, SMB

Pub # P1-111

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Effects of Bisphenol A on Male Mouse Fertility

Author String SP Singh, S Chouhan, J Prakash, S Yadav, Nk Agrawal, A Ghosh
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Body Increased occurrence of reproductive disorders has raised concerns regarding the impact of endocrine-disrupting chemicals on reproductive health. Bisphenol A (BPA) is a widespread estrogenic chemical used as a plasticizer. Exposure to BPA is strongly associated with adverse health effects on humans and laboratory animals.

The present study was undertaken to decipher the effects of BPA exposure, on fertility of male mice. A set of eight Swiss mice were injected with BPA intraperitoneally at a dose of 100[micro]g/kg/day for a period of two months. Normal saline was given to control group. Sperm count was carried out by collecting it from cauda region of epididymis. Blood samples were collected from treated and control group, and the testosterone level was measured by Radio immuno assay (RIA).

Females mated with male mice, that were exposed to BPA showed a 50% decrease in litter size. In control group number of pups varied from 10-12, whereas that in treated group it varied from 5-6. There were significant changes in sperm count along with hormonal imbalances in the mice exposed to BPA. Sperm count was found to be in the range of 1.2-2.4 million in control mice as compared to 0.54 -0.8 million in treated mice. The microscopic study of testes showed immense distortion of the sertoli cells and damaged seminiferous tubules in treated mice. The testosterone level in control group was 345-456 ng/dl and in treated group it was 156-262 ng/dl. The present study gives strong evidence to prove that even very low doses of BPA, as low as 100[micro]g/kg/day is capable of impairing male fertility, by lowering the sperm count, testosterone production, number of pups produced by these male mice and damaging the testes of treated animals.

Nothing to Disclose: SPS, SC, JP, SY, NKA, AG

Pub # P1-112

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title The Effects of Bisphenol A Exposure on G Protein Expression in the Hypothalamic-Pituitary-Gonadal Axis in Mice

Author String SA Sands, T Musick, JJ Leonard, JD Jacobson
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Body Several recent reports suggest that the development of secondary sexual characteristics is occurring earlier in girls. Changes in timing of such pubertal events in recent years suggest that factors other than genetics are involved, such as environmental exposure to endocrine disrupting chemicals. One such environmental chemical is bisphenol A (BPA), which is found in many common products such as food containers and baby bottles. BPA is detected in human tissue and organs such as the placenta as well as in maternal and fetal plasma. Prenatal BPA exposure is known to alter gene expression later in life through epigenetic mechanisms. The expression of secondary sexual characteristics is controlled by the hypothalamic and pituitary hormones GnRH, LH, and FSH. The actions of these hormones are mediated by cell surface receptors coupled to nucleotide binding proteins Gs alpha and Gq alpha, encoded by GNAS and GNAQ genes, respectively. Herein, we hypothesized that in vivo and in vitro BPA exposure would alter Gs alpha and Gq alpha mRNA expression in the hypothalamic pituitary gonadal axis. In vitro studies utilized the GnRH-secreting hypothalamic cell line GT1-7. In vivo studies involved prenatal treatment of pregnant mice with BPA (40 mcg/day) or vehicle (corn oil) began on gestational day 5, and continued every other day until parturition. On postnatal day 28, RNA was extracted from tissues and reverse transcribed into cDNA. RT-PCR was performed using an iCycler. Results from RT-PCR were normalized using the housekeeping gene GAPDH and expressed as a percentage of pooled mouse spleen cDNA. BPA exposure led to increased expression of Gs alpha mRNA (mean \pm S.D.: $62.25 \pm 19.43\%$ versus $42.25 \pm 10.68\%$; $p < 0.01$; $n = 4$) and Gq alpha mRNA ($77.45 \pm 33.66\%$ versus $48.57 \pm 21.83\%$; $p < 0.05$; $n = 4$) compared to vehicle in GT1-7 cells. Prenatal exposure to BPA led to increased expression of Gs alpha mRNA ($819.03 \pm 134.68\%$ versus $617.51 \pm 127.23\%$; $p < 0.05$; $n = 5$) and Gq alpha mRNA ($224.51 \pm 86.39\%$ versus $115.57 \pm 37.44\%$; $p < 0.05$; $n = 5$) compared to vehicle in the pituitary. Similar trends were observed in the ovary. These findings suggest that G protein mRNA expression in the hypothalamic pituitary gonadal axis can be altered following in vivo and in vitro exposure to BPA.

Sources of Research Support: Hall Foundation Trust.

Nothing to Disclose: SAS, TM, JJJ, JDJ

Pub # P1-113

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Perfluorooctanoic Acid Inhibits Fetal Rat Lung 11 β -Hydroxysteroid Dehydrogenase 1 Activity

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Body Glucocorticoids are critical for fetal lung development and function. The level of active glucocorticoid (corticosterone, CORT, in the rat) inside lung is increased by 11 β -hydroxysteroid dehydrogenase 1 (11 β HSD1) which activates biologically inert 11-dehydrocorticosterone (11DHC). Mutation of 11 β HSD1 has been shown to delay the lung maturation. Perfluorinated substances (PFASs) are environmental endocrine disruptors found in the tissues of humans and wildlife. The objective of the present study was to investigate whether PFASs directly inhibit fetal lung 11 β HSD1 activity. We screened five different PFASs including perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), potassium perfluorooctane sulfonate (PFOSK), potassium perfluorohexane sulfonate (PFHxSK) and potassium perfluorobutane sulfonate (PFBSK) in the inhibition of 11 β HSD1 activity in rat fetal lung microsomes. When rat lung microsomal enzymes were exposed to 250 [micro]M PFASs, the potencies for inhibition of 11 β HSD1 activity were determined to be PFOA (100% inhibition) >PFOSK (45%) = PFOS (42%) >PFHxSK (32%) >PFBSK (9%). IC₅₀ of PFOA in the inhibition of the enzyme is 24.3 [micro]M. The mode of inhibition of 11 β HSD1 is competitive for the 11DHC and uncompetitive for cofactor NADPH. In conclusion, the present study shows that PFOA is a potent inhibitor of rat fetal lung 11 β HSD1, and implies that inhibition of 11 β HSD1 may be a contributing factor to the delayed maturation of fetal lung.

Nothing to Disclose: LY, BZ, YC, R-SG

Pub # P1-114

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine-Disrupting Chemicals (1:30 PM-3:30 PM)

Title Smoking-Related Dyslipidemia and Inhibition of Apolipoprotein A-I Gene Expression by the Aryl Hydrocarbon Receptor

Author String E Naem, R Alcalde, M Gladysz, S Mesliniene, M Sheikh-Ali, MJ Haas, NC Wong, AD Mooradian
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Body Epidemiological studies have shown that smoking is a major risk factor for cardiovascular disease (CVD). There is an inverse association between plasma high-density lipoprotein cholesterol (HDLc) levels and the prevalence of CVD. Smokers have lower plasma concentrations of HDLc and apolipoprotein A-I (apo A-I) compared with nonsmokers suggesting that smoking may promote CVD by inhibiting apo A-I synthesis through the aryl hydrocarbon (AH) receptor. Treatment of HepG2 cells with the AH receptor agonists benzo(a)pyrene and CAY10854 inhibited mRNA and protein synthesis, while nicotine had no effect. Both benzo(a)pyrene and CAY10854 but not nicotine suppressed apo A-I gene promoter activity. Inhibition of apo A-I gene promoter activity was inhibited the AH receptor antagonist CAY10855 and by AH receptor-specific siRNA but not a control siRNA. Using a series of apo A-I gene promoter deletion constructs, the AH receptor-responsive region identified between nucleotides -325 and -186 (relative to the transcriptional start site, +1). This region contains a peroxisome proliferator-activated receptor alpha responsive region that is also negatively regulated by inflammatory cytokines such as interleukin-1 beta and tumor necrosis factor alpha. Benzo(a)pyrene had no effect on hepatic ATP binding cassette protein-A1 and scavenger receptor-B1 mRNA levels. These results suggest that smoking related hypoalphalipoproteinemia is promoted in part by polycyclic aromatic hydrocarbon binding to the hepatic AH receptor.

Sources of Research Support: Dean's Fund Research Grant from the University of Florida-Jacksonville.

Nothing to Disclose: EN, RA, MG, SM, MS-A, MJH, NCW, ADM

Pub # P1-115

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Growth Control, Thyroid Function & Diabetes (1:30 PM-3:30 PM)

Title Down-Regulation of Multiple Growth-Promoting Genes during Juvenile Growth Deceleration Is Associated with Decreasing Set1 Histone Methyltransferase Complex Formation and a Global Decline in Histone H3K4 Trimethylation

Author String JCK Lui, J Baron
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Body Body growth is rapid in early life but decelerates with age because proliferation is suppressed concomitantly in many tissues. The mechanisms responsible for this potent, widespread physiological suppression of cell proliferation are poorly understood. We recently showed evidence that this growth deceleration results from a growth-limiting genetic program that occurs simultaneously in multiple organs. This program involves the downregulation of many growth-promoting genes, including *Igf2*, *Mdk*, *Mycn*, and *Ezh2*, that are required for rapid proliferation in early life. The coordinate downregulation of these genes during juvenile life appears to be orchestrated by epigenetic mechanisms; in mice, from 1- to 4-wk of age, trimethylation of histone H3 at the lysine 4 residue (H3K4me3), a histone modification associated with gene activation, decreases in multiple genes of this program, in multiple organs. Furthermore, the overall level of H3K4me3 also decreases during juvenile growth deceleration, suggesting a global shift of chromatin toward a non-permissive state in these organs.

In the current study, we sought to identify the mechanisms that drive the change in chromatin structure. We hypothesized that the Set1 complex, which is the major histone methyltransferase responsible for trimethylation of H3K4, is downregulated with age. Using western blot, we found that in the Wdr5-Rbbp5-Ash2l core complex, which regulates the Set1 enzymatic activity, both Wdr5 and Rbbp5 were strongly downregulated from 1- to 4- and 8-wk of age in mouse liver, kidney, and lung. Quantitative real-time PCR showed that the mRNA levels of Wdr5 and Rbbp5 did not decrease with age, suggesting that their expression is regulated at the post-transcriptional level. Next, using co-immunoprecipitation, we found that the association of Wdr5, Rbbp5, and Ash2l in the core complex also decreased from 1- to 4- and 8-wk in liver, kidney, and lung, suggesting that decreased core complex formation might allow degradation of its components.

Taken together, our findings suggest that juvenile growth deceleration results from the downregulation of many growth-promoting genes and that this downregulation is orchestrated by a global decline in H3K4me3. This change in chromatin structure may result from a progressive posttranscriptional downregulation of the Wdr5-Rbbp5-Ash2l core complex, which is critical for the H3K4 methyltransferase activity of the Set1 complex.

Sources of Research Support: Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH.

Nothing to Disclose: JCKL, JB

Pub # P1-116

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Growth Control, Thyroid Function & Diabetes (1:30 PM-3:30 PM)

Title Evidence That Variation in Adult Body Size among Mammalian Species Is Achieved by Modulating the Pace of a Growth-Limiting Genetic Program

Author String A Delaney, JC Lui, G Rezvani, P Forcinito, V Padmanabhan, J Baron
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Body Body size varies enormously among mammalian species. In small mammals, body growth is typically suppressed rapidly, within weeks, whereas in large mammals, growth is suppressed slowly, over years, allowing for a greater adult size. We recently reported evidence that body growth suppression in rodents is caused in part by a juvenile genetic program that occurs in multiple tissues simultaneously and involves the downregulation of a large set of growth-promoting genes (1, 2). We hypothesized that this genetic program is conserved among mammalian species but its time course is evolutionarily modulated such that it plays out more slowly in large mammals, allowing for more prolonged growth. To test this hypothesis, we used microarray analysis to compare gene expression in kidney and lung of 1, 4, 8 wk-old mice; 1, 5 wk-old rats; and fetal day 90, 10-wk postnatal, and adult sheep. In all 3 species, we found a large set of genes downregulated with age (false discovery rate < 0.05, fold-change > 2) in both lung and kidney. The set of downregulated genes was similar in the 3 species; the number of overlapping genes between each pair of species was far greater than expected by chance ($P < 0.001$, Chi square test), indicating evolutionary conservation of the program. To compare the pace of the program, we studied the 25 genes that were downregulated in both organs of all 3 species. In mouse, the greatest declines occurred by 4 wk of age (26 ± 3 kidney; 30 ± 3 , lung; % expression relative to 1 wk; mean of 25 genes \pm SEM), followed by a slower decline (16 ± 2 , kidney; 26 ± 2 , lung; % expression at 8 wk relative to 1 wk). In rat, the declines occurred more gradually ($P = 0.025$, kidney; $P < 0.001$, lung) than in mice (32 ± 2 , kidney; 40 ± 2 , lung; % expression in 5-wk rats relative to 1-wk). In sheep, the declines appeared to occur even more slowly; large declines occurred between 90 d gestation (a greater age post-fertilization than 1-wk-old mouse or rat) and 10 wks of age (35 ± 2 kidney; 44 ± 4 , lung; % expression relative to 90 d gestation), and this decline continued ($P = 0.001$, kidney; $P < 0.001$, lung) after 10 w (29 ± 2 , kidney; 34 ± 4 , lung; % expression in adult relative to 90 d gestation). Additional time points are being analyzed to confirm this pattern. The data suggest that a growth-limiting genetic program is conserved among mammalian species but that its pace is modulated to allow more prolonged growth and therefore greater adult body size in large mammals.

- (1) Finkelstein GP et al., *Endocrinology* 150:1791-800, 2009
(2) Lui JC et al., *FASEB J.* 24:3083-92, 2010

Sources of Research Support: Intramural Research Program, National Institute of Child Health and Human Development, NIH.

Nothing to Disclose: AD, JCL, GR, PF, VP, JB

Pub # P1-117

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Growth Control, Thyroid Function & Diabetes (1:30 PM-3:30 PM)

Title Changes in MicroRNA Expression during Juvenile Growth

Author String F Kamran, AC Andrade, G Rezvani, O Nilsson, J Baron, JC Lui
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Body Body growth is rapid in young children, but with increasing age, growth gradually slows and eventually ceases in multiple organs because of a progressive decline in cell proliferation, which begins in early life. We previously showed evidence that this decline in proliferation is caused in part by coordinated postnatal downregulation of a large set of growth-promoting genes (Endocrinology 150:1791-800, 2009; FASEB J 24:3083-92, 2010). We hypothesized that microRNAs help orchestrate this juvenile genetic program. MicroRNAs are endogenous short non-coding RNAs that regulate gene expression by binding to multiple messenger RNAs, consequently blocking translation or stimulating mRNA degradation. MicroRNAs play an important role in regulating a variety of cellular processes, including proliferation. To explore the possibility that changes in microRNA levels help coordinate this genetic program, we compared microRNA expression in juvenile mice at different ages, as growth slows. MicroRNA microarray (Agilent Mouse Genome VI microRNA arrays, targeting 567 microRNAs) was performed in mouse kidney and lung tissue at 1 and 6 weeks of age (n=6 per group) and analyzed using GeneSpring software. A significant change with age was defined by t-test with Benjamini-Hochberg false discovery rate < 0.05 and fold change > 1.3. Because the juvenile genetic program occurs in multiple organs, we focused on the 8 microRNAs that showed concordant upregulation (microRNAs 133a, 328 and 7a) or downregulation (microRNAs 106b, 351, 450b-3p, 409-3p and 376a) with age in both organs. Real-time PCR of the mature microRNAs (normalized to Sno234 expression) was used to verify our microarray results. To date we have examined 3 microRNAs in lung at ages 1, 4 and 8 weeks. The results confirmed significant upregulation of microRNA-133a (P = 0.003) and microRNA-7a (P = 0.001) with age. MicroRNA-106b expression declined but did not reach significance (P = 0.054). In conclusion, we have identified specific microRNAs that show changes in expression during juvenile life in both mouse kidney and lung. Whether these microRNAs help coordinate the juvenile regulation of multiple growth-regulating genes and thus drive growth deceleration remains to be determined.

Nothing to Disclose: FK, ACA, GR, ON, JB, JCL

Pub # P1-118

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Growth Control, Thyroid Function & Diabetes (1:30 PM-3:30 PM)

Title Monosomy X and Disruption of Genomic Imprinting -- Aberrant Allele-Specific Expression at the IGF2 Locus

Author String HY Hougen, C Cheng, CA Bondy
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Body Turner Syndrome (TS) is caused by complete or partial monosomy for the X chromosome and is associated with growth retardation and primary ovarian failure. To understand how monosomy X produces such a profound phenotype, we compared peripheral blood leukocyte (PBL) gene expression profiles in 45,X (N=26) and 46,XX (N=10) women, using Affymetrix genechip U133 plus2. We found that transcript levels for genes regulated by genomic imprinting are selectively altered. Specifically, expression of 139/208 (66%) of known and predicted imprinted genes, including IGF2, H19, IGF2R and DLK1, is selectively altered in 45,X females. This effect was independent of the parental origin of the single X chromosome. In contrast, gene expression profiling data for trisomies X, 18, and 21 from an in silico analysis with the exact same statistical criteria showed no selective alteration of imprinted gene expression, suggesting that the effect is not due to aneuploidy per se. To determine whether monosomy X alters imprinting mechanisms, we analyzed allele-specific expression for IGF2 using RT-PCR and restriction fragment length polymorphisms for two informative single nucleotide polymorphisms in age-matched 45,X patients with TS (N=23) and 46,XX healthy volunteer controls (N=13). The 45,X individuals had a higher frequency of biallelic expression compared with 46,XX individuals at two loci from exon 9 of IGF2. Similar analyses of other imprinted genes are in progress. This is a novel discovery showing altered expression of genomically imprinted autosomal genes in women with X chromosome monosomy associated with loss of allele-specific expression of the IGF2 gene cluster. Previous clinical studies have suggested that growth restriction in TS is not entirely explained by haploinsufficiency for the pseudoautosomal X-linked SHOX gene, and several studies have confirmed that adult height in girls with TS is related to maternal but not paternal height. Since genomic imprinting is involved in normal growth and development, the current data suggest that the altered imprinting of IGF2 and other growth related genes may contribute to the growth deficit in TS.

Sources of Research Support: National Institutes of Health Intramural Research Program.

Nothing to Disclose: HYH, CC, CAB

Pub # P1-119

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Growth Control, Thyroid Function & Diabetes (1:30 PM-3:30 PM)

Title Pleiotropy Involving Skeletal, Vascular, and Reproductive Phenotypes in Recombinant Congenic Mice

Author String J Kristianto, JS Fisher, MG Johnson, CY Ooi, Z Wang, SJ Litscher, N Chesler, RD Blank
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Body Pregnancy complications result in devastating outcomes, including IUGR, preeclampsia and fetal death *in utero*. Epidemiological data suggests that children of IUGR suffer developmental problems and exhibit increased risk of metabolic abnormalities as adults. Placental insufficiency has been suspected as one of the main causes of these pregnancy complications. Our laboratory has identified a pleiotropic (a single gene affecting multiple traits) quantitative trait locus (QTL) on mouse chromosome 4 that modulates skeletal modeling, apparently in response to mechanical loading. We hypothesize that the QTL-mediated modeling differences in response to mechanical loading are shared between bone and blood vessels. To test this hypothesis, we have undertaken a series of studies in HcB-8 (low modeling) and HcB-23 (high modeling) recombinant congenic mice, which harbor the alternative genotypes for the chromosome 4 QTL. Recombination during the construction of these strains limits the QTL region to approximately 6.5 megabases. HcB-8 litters are smaller than HcB-23 litters (4.4 ± 1.8 v 5.8 ± 2.0 , $p < 10^{-3}$). HcB-8 pups are smaller at birth than HcB-23 pups (1.2 ± 0.2 g v 1.5 ± 0.2 g, $p < 10^{-20}$). Of 4 pregnant HcB-8 females examined, all had at least 1 resorbed embryo, while no resorbed embryos were observed in pregnant HcB-23 mice. Reasoning that placental insufficiency could account for these differences, we studied carotid artery mechanics in HcB-8 and HcB-23 mice. HcB-8 carotid arteries have a smaller diameter at 90 mm Hg than HcB-23 carotids ($p=0.026$). Moreover, the HcB-8 arteries are less compliant to increases in luminal pressure ($p=0.036$). Because the gene encoding endothelin converting enzyme 1 (*Ecel*) resides within the candidate QTL interval, we examined its expression in HcB-8 and HcB-23 by real-time PCR and western blotting. At the mRNA level, HcB-23 expresses nearly 3-fold more *Ecel*, $p < 10^{-3}$, with the protein data showing a similar pattern of abundance. In prior published work, we have shown that the chromosome 4 QTL affects multiple aspects of bone size, shape, and mechanical performance. Data presented here indicate that the QTL's pleiotropy extends beyond the skeletal system. Further, they suggest that *Ecel* is a strong candidate gene for the QTL. We propose that mechanically-induced modeling is the common mechanism linking the various traits controlled by the chromosome 4 QTL.

Nothing to Disclose: JK, JSF, MGJ, CYO, ZW, SJL, NC, RDB

Pub # P1-120

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Growth Control, Thyroid Function & Diabetes (1:30 PM-3:30 PM)

Title Cloning a Prop1-Binding Factor That Regulates Pit-1 Gene Expression

Author String Y Okimura, Y Sugiyama, D Yamamoto, M Shibahara, N Ikeshita, T Maki, EH Herningtyas, K Iida, G Iguchi, Y Takahashi, H Kaji, K Chihara
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Body Prop1 is a transcription factor that stimulates initial activation of Pit-1 gene. C-terminal region of Prop1 is assumed to be a transactivation domain (TAD) that interacts with cofactors. Some patients with combined pituitary hormone deficiency (GH, PRL and TSH deficiency) have mutations in the TAD and the phenotype is similar to that of patients with Prop1 mutation in DNA-binding domain. Although this indicates that interaction with cofactors is important for the function of Prop1, Prop1 cofactors have not been identified, yet. In the present study, we aimed to identify Prop1 cofactors from human brain cDNA library to analyze their functions. We cloned several candidate proteins that may bind to Prop1 using yeast two-hybrid assay. Seven colonies contained amino-terminal enhancer of split (AES) out of total eighteen colonies in the two-hybrid assay and we first analyzed the AES function. Immunoprecipitation assay showed that Prop1 bound to AES when overexpressed in Cos cells. AES Q domain, N-terminal domain, was identified to be an interacting part with TAD in mammalian two-hybrid assay using TAD as bait. Deletion of leucine zipper-like motif (LZL) of Q domain indicated that the LZL motif was essential for the interaction with TAD. Prop1 stimulated Pit-1 Luc reporter gene in GH3 cells and this activation was partly inhibited by AES. On the other hand, AES alone did not influence Pit-1 reporter gene activation. It is reported that AES is present in Rathke's pouch, AES knockout mice result in Rathke's pouch abnormalities, AES is a modest repressor of pituitary growth and Prop1 dysfunction also causes abnormal pituitary development. The interaction of Prop1 and AES shown in the present study might be involved in the abnormal development of Rathke's pouch and pituitary.

Nothing to Disclose: YO, YS, DY, MS, NI, TM, EHH, KI, GI, YT, HK, KC

Pub # P1-121

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Growth Control, Thyroid Function & Diabetes (1:30 PM-3:30 PM)

Title BAC Recombineering to Understand the Role of the Alternative Promoter in the Regulation of Prolactin Expression

Author String R Awais, A McNamara, CV Harper, A Adamson, DG Spiller, S Semprini, JJ Mullins, JRE Davis, MRH White
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Body Many genes are controlled by alternative promoters, including the prolactin (PRL) gene, which in man is expressed at extra-pituitary sites in addition to the pituitary. The extra-pituitary expression of PRL is controlled by an alternative promoter located 5.8Kbp upstream of the pituitary transcription start site. Previous studies using short promoter fragments may be misleading, since the human (h) PRL genomic locus has many conserved regions far-upstream of the transcriptional start site. To study the function of the alternative promoter, we engineered a bacterial artificial chromosome (BAC) expressing Luciferase (Luc) under the control of a 163Kb hPRL genomic fragment (1), deleting the entire 5kbp pituitary promoter by BAC-recombineering, leaving intact the upstream exon 1a and alternative promoter. The alternative splice acceptor site was reinserted after mutating three adjacent Pit-1 binding sites. This alternative PRL promoter BAC- Luc reporter gene (AP-BAC-Luc) was used to generate stably-transfected rat pituitary GH3 and Jurkat lymphoblastoid cell lines, representing cellular models of pituitary and extra-pituitary sites of expression. Surprisingly, AP-BAC-Luc was active in both GH3 and Jurkat cell lines, with greater signal measured in pituitary cells. PRL-regulating stimuli, including basic Fibroblast Growth Factor (FGF2), Forskolin and Phorbol-12-myristate-13-acetate (PMA), induced an increase in AP-BAC-Luc expression in the GH3 recombinant stable clones (similar fold-activation to that seen from the pituitary PRL promoter). Oestrogen and TNF α failed to induce AP-BAC-Luc expression, in contrast to their strong induction of PRL pituitary promoter activity in GH3 cells. Real-time luminescence imaging of living cells showed dynamic patterns of expression, as seen with pituitary promoter constructs, indicating that cyclical transcriptional function is shared by the alternative promoter. These results provide new insights into how alternative promoters differentially or coordinately regulate PRL expression. Our data indicate that both promoters share activity within the same cell, and both display cyclical function. In this context, the AP-BAC-Luc generated in this study offers a powerful model approach to understand mechanisms responsible for differential activation of alternative promoters in alternative sites of gene expression.

(1) Semprini et.al Molecular Endocrinology(2009), 23:529-538

Nothing to Disclose: RA, AM, CVH, AA, DGS, SS, JJM, JRED, MRHW

Pub # P1-122

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Growth Control, Thyroid Function & Diabetes (1:30 PM-3:30 PM)

Title Thyrotropin-Releasing Hormone Regulates the Transcription of Fibroblast Growth Factor 21 Gene in Pancreas

Author String J Garay Guerrero, N Shibusawa, A Ozawa, K Hashimoto, T Satoh, M Yamada, M Mori
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Body Thyrotropin-releasing hormone (TRH) has been identified in many systemic organs including the pancreas(1) (2). The TRH-deficient mice (TRHKO) showed characteristic tertiary hypothyroidism and also developed hyperglycemia associated with impaired insulin secretion in response to glucose (3). We conducted a microarray study with islets of Langerhans isolated from the TRHKO pancreas and found the expression of fibroblast growth factor 21 (FGF21) mRNA to be one of the most significantly decreased in TRHKO, which was not restored by thyroid hormone replacement. In vitro experiments revealed that the FGF21 mRNA and protein levels were increased in a mouse b cell line that contains TRH receptors, Beta TC-6, in the presence of TRH in a time- and dose- dependent manner. In the transfection reporter assay, TRH stimulated the promoter activity of the human FGF21 gene in the chinese hamster ovarian (CHO) cells that expressed human TRH receptors, but not in the cells without expression of TRHR. In addition, the inhibition of MAPK pathway with PD98059 reduced the TRH-induced stimulation of the human FGF21 promoter. Deletion analysis of the promoter region demonstrated a 45 bp between -277 bp and -322 bp from the transcription start site to be responsible of this effect. Furthermore, an electrophoretic mobility shift assay (EMSA) revealed that the middle 20 bp of the above 45 bp was the responsible region. These findings demonstrated that TRH regulates transcription of the FGF21 gene through mitogen-activated protein kinase (MAPK) pathway in the pancreas in a time and dose dependent manner.

(1) Morley et al., Biochem Biophys Res. Comm 1977; 79:314
(2) Martino et al., Proc Natl Acad Sci U S A 1978.; 75(9):4265
(3) Yamada et al., Proc Natl Acad Sci U S A 1997;94(20):10862

Nothing to Disclose: JGG, NS, AO, KH, TS, MY, MM

Pub # P1-123

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Growth Control, Thyroid Function & Diabetes (1:30 PM-3:30 PM)

Title Genetic Variation in the *FGF23* Gene Associates with Phosphate Homeostasis and Bone Turnover in Healthy Children and Adolescents

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Body Fibroblast growth factor 23 (FGF23), synthesized by osteoblasts and osteocytes, is part of the hormonal bone-parathyroid-kidney axis, which is modulated by PTH, 1,25(OH)₂-vitamin D, dietary intake and serum phosphate (Pi) levels. Several hereditary disorders that exhibit inappropriately high serum FGF23 levels are associated with phosphate wasting and impaired bone mineralization. In the present study we investigated the genetic variation in the *FGF23* gene and its association with parameters of phosphate homeostasis and bone health in Finnish children and adolescents. This school-based study included 183 healthy children and adolescents (110 girls, 73 boys) aged 7-19 years, who were assessed for bone health and its determinants. The genetic variation was detected by direct sequencing *FGF23* exons 2 and 3 with flanking intronic regions. The concentrations of plasma/serum Ca, Pi, 25-hydroxyvitamin D (25-OHD), PTH and the bone formation marker PINP, as well as urine Pi and creatinine concentrations were determined. BMD was assessed with DXA (Hologic Discovery A). Statistical analyses were performed with SPSS. We detected three different *FGF23* polymorphisms: c.212-37insC in intron 1 and two SNPs in exon 3, rs7955866 (p.T239M) and rs11063112 (p.P241P). Genotypes among the 183 subjects were for c.212-37insC +/- (78%) and -/C (22%); for rs7955866 G/G (80%) and G/A (20%); and for rs11063112: A/A (47%), A/T (45%) and T/T (8%). The c.212-37insC was significantly associated with lower P-Pi (p=0.021), U-Pi (p=0.013) and S-PINP (p=0.026) but not with U-Pi/U-Crea or with lumbar spine, femur or whole body BMD Z-score. The exon 3 SNPs did not associate with any of the measured parameters. The observed association between *FGF23* polymorphism and P-Pi and S-PINP and suggests that c.212-37insC genotype may play a role in mineral homeostasis and bone remodeling.

Sources of Research Support: Academy of Finland; Folkh[auml]lsan Research Foundation; Sigrid Juselius Foundation.

Nothing to Disclose: MP, CML, RM, HTV, OM

Pub # P1-124

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Growth Control, Thyroid Function & Diabetes (1:30 PM-3:30 PM)

Title Altered Gene Expression in Insulin-Degrading Enzyme (IDE) Knock-Out (KO) Mice

Author String FG Hamel, J Eudy, F Qiu, G Siford, RG Bennett
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Body IDE is a ubiquitously expressed zinc metalloprotease which is responsible for initiating the degradation of insulin, as well as other peptides. IDE KO mice have elevated insulin levels and are glucose intolerant. Thus, altered insulin processing has adverse effects on insulin signaling. We compared gene expression in liver from KO and WT mice. Mice were fasted overnight, liver samples taken and placed in RNAlater[reg]. Total RNA was purified with the PureLink[trade] RNA kit, treated with DNase, and was visualized on agarose gels to verify integrity. Gene expression was analyzed with Affymetrix GeneChip[reg] Mouse Genome 430 arrays. The differential expression analysis was conducted with the limma package in Bioconductor. Of 38 genes found to be changed at the 0.0001 significance level between KO and WT, 35 had a 2-fold or greater change. This set of 38 genes was associated with a false discovery rate of under 5.1%. The differentially expressed genes represented areas of potential direct relevance to insulin action, signal transduction, or metabolism. The upregulated genes, the ratio of KO/WT, and the p values include: phosphoinositide-3-kinase adaptor protein 1 8.94, $p=5.51E-09$; thyroid hormone receptor beta, 5.06, $p=6.10E-06$; glucokinase, 2.79, $p=2.17E-06$; mitogen activated protein kinase 8 (JNK1), 2.68, $p=6.31E-05$; and dual specificity phosphatase 1, 2.68, $p=3.88E-06$. Expression of a number of genes was also decreased in KO compared to WT: guanylate cyclase soluble subunit alpha-3, 0.55, $p=5.71E-05$; cAMP specific phosphodiesterase 4B, 0.32, $p=4.56E-05$; and ribosomal protein S9, 0.04, $p=2.15E-10$. Upregulation of glucokinase would directly alter insulin-mediated control of glucose metabolism, while downregulation of ribosomal protein S9 could alter insulin-mediated control of protein synthesis. Phosphoinositide-3-kinase adaptor protein, JNK, dual specificity phosphatase 1, guanylate cyclase soluble subunit alpha-3, and cAMP specific phosphodiesterase 4B are important for signal transduction. Thyroid hormone receptor beta upregulation could potentially induce metabolic changes that oppose insulin action. These findings indicate that IDE has significant effects on cellular metabolism beyond the simple degradation of insulin and other peptides. Inhibition of IDE by fatty acids or nitric oxide may have similar effects as found in the KO mice. The genes affected will help direct future studies to determine the exact effect of IDE on insulin signal transduction.

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Nothing to Disclose: FGH, JE, FQ, GS, RGB

Pub # P1-125

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Growth Control, Thyroid Function & Diabetes (1:30 PM-3:30 PM)

Title The Transcription Factor *SOX3* Is a Putative Target Gene of GATA3: Relevance to Hypoparathyroidism and Parathyroid Development

Author String KU Gaynor, IV Grigorieva, MA Nesbit, RV Thakker
University of Oxford, Oxford, UK

Body An interstitial deletion-insertion [del(X)(q27.1) inv ins (X;2)(q27.1;p25.3)], that is located 67 kb downstream of the gene encoding the transcription factor *SOX3*, has been reported in patients with X-linked recessive hypoparathyroidism, thereby indicating involvement of a non-coding region abnormality that may alter *SOX3* expression in the etiology of this disorder of parathyroid development (1). We hypothesized that the transcription factor GATA3 may regulate *SOX3* expression as: (i) *GATA3* mutations result in the hypoparathyroid, deafness and renal dysplasia syndrome (2); (ii) GATA3 has been reported to regulate expression of the parathyroid specific transcription factor *GCMB/2*, mutations of which lead to isolated hypoparathyroidism (3); and (iii) *Gata3*, *Gcmb/2* and *Sox3* are reported to be expressed in embryonic mouse parathyroids (1,4,5). As a first step towards investigating this hypothesis we undertook an *in silico* search for GATA3 binding motifs in the *SOX3* promoter region, -427 to +286 relative to the transcription start site (TSS) (6), using the transcription factor binding site databases Consite, Match, MatInspector, TFBind and TFSearch. This identified 7 putative GATA3 binding motifs (*a* to *g*) in the *SOX3* promoter region, at positions -243 to -246, -223 to -220, -192 to -189, -69 to -66, -51 to -48, +185 to +188 and +267 to +270, respectively, relative to the TSS. Direct GATA3 protein and *SOX3* DNA interaction at these sites was investigated by electrophoretic mobility shift assays (EMSAs), which used nuclear protein extracts prepared from COS7 cells transfected with a GATA3 expression vector and radiolabeled double-stranded *SOX3* DNA oligonucleotides containing each of the 7 putative GATA3 binding motifs. These EMSAs revealed likely GATA3 protein binding to motifs *d* and *g*, and to determine the specificity of this binding by detecting the presence of GATA3 in these complexes, EMSAs with a GATA3 antibody were used (supershift EMSAs). This revealed that only motif *g*, located at +267 to +270, was specifically bound by GATA3. Thus, our results reveal that the *SOX3* promoter has a GATA3 binding site, and that GATA3 and *SOX3* may form part of a transcription cascade with a potential role in regulation of parathyroid development.

- (1) Bowl MR et al., J Clin Invest 2005; 115:2822
- (2) Van Esch et al., Nature 2000; 406:419
- (3) Bowl MR et al., Hum Mol Genet 2010; 19:2028
- (4) Grigorieva et al., J Clin Invest 2010; 120:2144
- (5) Gordon J et al., Mech Dev 2001; 103:141
- (6) Kovacevic Grujicic et al., Gene 2005; 344:287

Nothing to Disclose: KUG, IVG, MAN, RVT

Pub # P1-126

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Growth Control, Thyroid Function & Diabetes (1:30 PM-3:30 PM)

Title The DNA Binding Patterns of Thyroid Hormone Receptor

Author String S Ayers, P Webb, J Baxter
Methodist Hospital Research Institute, Houston, TX

Body Thyroid hormone mediates a variety of physiological effects through thyroid hormone receptors (TR). These receptors regulate transcription by binding to elements throughout the genome DNA as homodimers, or as heterodimers with other nuclear hormone receptors. In this study, we have performed a comprehensive analysis of TR binding sites in the genome using a novel Chromatin IP-Sequencing strategy, employing a biotinylated epitope tag (BioChIP-Seq). Using this strategy, we have discovered that thyroid hormone receptor binds to a more diverse set of sequences than expected and exhibits unexpected changes in response to hormone binding. The results of this study add to our understanding of thyroid hormone regulation of target genes through DNA binding events.

Disclosures: JB: Consultant, Karo Bio AB; Stock Owner, Kar Bio AB. Nothing to Disclose: SA, PW

Pub # P1-127

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Insulin-Like Growth Factor-I Inhibits Growth Hormone Expression Via Disruption of the PIT-1/CREB-Binding Protein Complex on the Proximal Growth Hormone Promoter

Author String CJ Romero, S Radovick
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Body **Introduction:** Circulating insulin-like growth factor-1 (IGF-I) plays a role in negative feedback of growth hormone (GH) gene expression and secretion; however, the mechanism of this feedback is poorly understood. Appropriate GH gene expression requires binding of transcription factor Pit-1 to the GH promoter and the Pit-1 dependent cofactor, cAMP response element binding protein (CREB) binding protein (CBP). We investigated the effect of IGF-I receptor (IGF-IR) signaling on Pit-1 and CBP using the MtT/S somatotroph cell line. We hypothesized that IGF-1R mediated phosphorylation of CBP disrupts the PIT-1/CBP complex and decreases GH gene expression. Furthermore, we tested this hypothesis *in vivo* using the somatotroph IGF-1R knockout (SIGFRKO) mouse model, which lacks IGF-1 feedback specifically in the somatotroph.

Methods and Results: In order to demonstrate IGF-1's inhibitory action on the MtT/S somatotroph cell line, a 2.7 bp mouse GH promoter construct, inserted into a luciferase reporter, was transfected into cells. IGF-1 treatment (30nM) after 24 hours demonstrated a 22.2% relative decrease in luciferase expression ($p \leq 0.001$). At 72 hours post IGF-1 treatment, we noted a maximal response (42.8% decrease, $p \leq 0.001$); although significant inhibition persisted at 96 hrs ($p \leq 0.001$). Previous data suggests IGF-1 increases CBP phosphorylation, but does not affect PIT-1 binding to the GH promoter. Luciferase expression in MtT/S cells transfected with wild type CBP demonstrated 50% inhibition after IGF-1 treatment ($p \leq 0.001$). Transfection of a mutant CBP (S436A) construct, which cannot be phosphorylated, demonstrated no difference in luciferase expression after IGF-1 treatment. We concluded that IGF-1 phosphorylation of CBP disrupts the PIT-1/CBP complex on the GH promoter. Finally, *in vivo* chromatin immunoprecipitation studies were performed using pituitary extracts from SIGFRKO and control mice. Control mice demonstrated decreased binding of CBP after IGF-1 treatment, but no change was seen in SIGFRKO mice; these data support the *in vitro* data that IGF-1R signaling disrupts the PIT-1/CBP complex.

Conclusion: We propose a novel mechanism of inhibition by IGF-1 in the somatotroph through disruption in the PIT-1/CBP complex on the GH proximal promoter. The SIGFRKO mouse model adds further support by demonstrating that loss of IGF-1R signaling in the somatotroph preserves the interaction of PIT1 and CBP on the GH promoter and results in increased GH gene expression.

Nothing to Disclose: CJR, SR

Pub # P1-128

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Unbound/Bio-Available IGF-I Enhances Somatic Growth

Author String H Jasper, H Domene, L Karabatas, C Guida, S Yakar
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Body IGF-I bioactivity is modulated by its binding to serum and tissues IGF binding proteins (IGFBPs). Des1-3-IGF-I (DES) is an IGF-I analog with normal affinity for the IGF-I receptor but much lower affinity to IGFBPs. To determine whether IGF-I binding to IGFBPs is necessary to facilitate normal growth, we generated a knock-in mouse model of DES-IGF-I (KID), where the native *Igf1* gene was replaced by *des-Igf1*, and therefore expressed under the endogenous *Igf1* promoter. IGF-I concentrations in serum of KID mice, measured by radio-immunoassay (National Hormone and Peptide Program, NIH), were reduced significantly as compared to controls. Similarly, IGFBP-3 levels reduced by ~40%, likely due to decreased protein stability in the absence of binding to IGF-I, while the levels of the acid-labile subunit (ALS) remained unchanged. Formation of ternary complexes (IGF-I/IGFBP-3/ALS) in serum of KID mice was significantly reduced when WT-IGF-I was used as a tracer, (28.8±3.9 vs 14.6±2.0, % total binding, p< 0.01). Likewise, bound IGF-I cpm/total cpm was significantly reduced in KID mice (57.23±3.76 vs 29.26±4.29, p=0.0008) when DES IGF-I was used as tracer. Despite significant reductions (60%) in serum IGF-I levels and reduced IGF-I ternary complex formation, KID mice exhibited increased body weight and body length in both males and females as compared to controls. We found that not all organs responded equally to DES-IGF-I. Relative weights (% BW) of kidney, pancreas, uterus and ovaries increased at all ages studied, while other tissues showed no difference when compared to controls. In summary, despite lower concentrations in serum, Des-IGF-I was more potent in promoting growth than native IGF-I. The lower affinity of DES for IGFBPs, resulting in a reduction of bound-IGFs in KID mice, suggests that an increase in bioactive DES (free plus that loosely bound to IGFBPs) at the target organ is responsible for increased body size and selective organ growth in KID mice. The KID model supports the important roles of IGFBPs in modulating IGFs bioavailability.

Sources of Research Support: NIH Grants AR054919 & AR055141 to SY.

Nothing to Disclose: HJ, HD, LK, CG, SY

Pub # P1-129

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Diet, Sex and Genotype Interactions in Mice Heterozygous for the IGF-I Receptor

Author String N Garg, S Thakur, ML Adamo
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Body Fat in the body can either come from de novo lipogenesis or from consumption of a high fat diet (HFD). Long term HFD feeding is accompanied with alterations in gene expression of enzymes involved in lipid and glucose metabolism. Interaction of dietary fat with sex hormones is a field poorly investigated. Studies in mice have demonstrated that estrogen protects females against diet-induced obesity thereby exerting protective effects against the metabolic syndrome and cardiovascular disease. Thus studying sex and diet interactions are essential.

Preliminary studies in our lab using the *Igflr*^{+/-} mice demonstrate insulin resistance and increased expression of lipogenic enzymes in the aged male mice associated with reduced IGF-1 signaling. We wanted to investigate whether HFD feeding to 19months *Igflr*^{+/-} mice accelerated insulin resistance which was observed at 25months in males and its response in females which were insulin sensitive at 25months. Studying the *Igflr*^{+/-} mice model on a HFD led us to dissect diet, genotype and sex interactions in these mice.

Our results demonstrate genotype interactions in aged female on a HFD. Female *Igflr*^{+/-} mice became more susceptible to insulin resistance compared to WT, had hyperinsulinemia and increased expression of liver fatty acid synthase and fatty acid translocase CD36. In contrast, the aged male WT and *Igflr*^{+/-} mice on HFD acquired a similar degree of insulin resistance. Male WT as compared to female WT had hyperglycemia, hyperinsulinemia, glucose intolerance and insulin resistance. Males of both genotypes had higher serum triglycerides, increased expression of lipogenic genes in liver and muscle and reduced expression of liver fatty acid oxidation genes and CD36.

The gender differences observed on HFD could originate from interactions between sex hormones and metabolic pathways involved in maintaining glucose and lipid homeostasis. Higher levels of estrogen in the aged female WT relative to the *Igflr*^{+/-} mice could contribute to the protective effect against HFD-induced insulin resistance. However, measuring levels of estrogen and exogenously administering estrogens to female *Igflr*^{+/-} mice to determine whether the insulin resistance is reverted, remains to be determined. Hence this study for the first time examines the influence of sex and *Igflr* heterozygosity on the response of carbohydrate and lipid metabolism to dietary fats.

Sources of Research Support: Translational Science Training Grant 129595 awarded to NG;; R01 AG026012 awarded to MLA.

Nothing to Disclose: NG, ST, MLA

Pub # P1-130
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)
Title The Relative Contributions of Endocrine and Paracrine/Autocrine IGF-I to Mouse Postpubertal Growth in GH-Deficient Mice with Hepatic JAK2 Disruption
Author String SM Nordstrom, JL Tran, BC Sos, K-U Wagner, EJ Weiss
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Body The relative contributions of circulating and locally-produced IGF-1 in growth remain controversial. The majority of circulating IGF-1 is produced in the liver, and numerous mouse models have been developed to test the role of liver-derived IGF-1. A common drawback to these models is that elimination of circulating IGF-1 disrupts a negative feedback pathway, resulting in unregulated GH secretion. We generated a mouse with near total abrogation of circulating IGF-1 by disrupting the GH signaling mediator, JAK2, in hepatocytes. We crossed these mice, termed JAK2L, to GH-deficient "litter" (Lit) mice to compare growth of the compound mutant (Lit-JAK2L) and its relevant control (Lit-Con). 30 day old Lit-JAK2L and Lit-Con mice were treated with equal amounts of GH for 26 days, such that the only difference between the two groups is in hepatic GH signaling (n=15-18). GH-treated Lit-JAK2L mice had large reductions in liver *Igf1* and *Igfals* ($P < 0.0001$), but normal *Igfbp3* expression vs GH-treated Lit-Con mice. This translated to a 93% reduction in circulating IGF-1 in Lit-JAK2L mice ($P < 0.0001$). Both groups treated with GH gained weight compared to untreated mice, but Lit-JAK2L mice had a 21% reduction in final weight vs Lit-Con ($P < 0.05$). Similarly, analysis by DEXA showed that lean mass increased with GH in both groups compared to untreated mice, but Lit-JAK2L mice had a 12% reduction in final lean mass vs Lit-Con ($P < 0.01$). In contrast to their differences in weight, Lit-Con and Lit-JAK2L mice had equivalent increases in tip-to-tail and femoral length with GH treatment. Both groups also increased bone mineral density (BMD) with GH-treatment, but Lit-JAK2L mice had significantly reduced skeletal and femoral BMD vs Lit-Con ($P < 0.05$). In addition, GH increased spleen and kidney mass in Lit-Con mice ($P < 0.01$), but had no effect in Lit-JAK2L mice. There were no differences between untreated Lit-Con and Lit-JAK2L mice in any measures. Taken together, this study shows that hepatic, GH-dependent production of circulating IGF-1 has significant and non-redundant roles in GH-mediated acquisition of lean mass, BMD, spleen mass, and kidney mass; however, skeletal and femoral length is dependent upon or compensated for by locally-produced IGF-1. This model presents a major advancement in defining the roles of endocrine vs paracrine/autocrine IGF-1 in growth and metabolism and these findings will ideally aid in the development of improved treatments for growth disorders.

Sources of Research Support: UCSF Liver Center pilot/feasibility grant P30 DK026743 awarded to EJW; NID Grant CA117930 awarded to KW; NHLBI training grant awarded to SMN.

Nothing to Disclose: SMN, JLT, BCS, K-UW, EJW

Pub # P1-131

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Elevated Endogenous GH/IGF-I, Due to Somatotrope-Specific Loss of Both IGF-I and Insulin Receptors, Improves Glucose Clearance, Despite Insulin Resistance

Author String MD Gahete, J Cordoba-Chacon, CV Anadumaka, Q Lin, CR Kahn, JC Bruning, RM Luque, RD Kineman
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Body Growth hormone (GH)-promoter driven Cre recombinase mice (rGHpCre) were backcrossed with mice harboring loxP-modified IGF-I receptor (Igf1rfl/fl) and insulin receptor (Insrfl/fl) genes, to obtain somatotrope-specific knockout of both Igf1r and Insr (KO). Gender-dependent differences in reduction of pituitary Igf1r and Insr mRNA and protein of KO mice (Cre+/-,Igf1rfl/fl,Insrfl/fl), compared to controls (Cre-/-,Igf1rfl/fl,Insrfl/fl), was consistent with the percentage of somatotropes reported in male/female pituitaries. GH levels were dramatically elevated and associated with a significant increase in total IGF-I. Of note, the changes in GH/IGF-I observed in these double KO mice were much more pronounced than that previously reported in somatotrope-specific Igf1r KO mice¹, demonstrating insulin, as well as IGF-I, is important in the negative feedback regulation of somatotrope function. Given ~10% of lactotropes in rGHpCre mice express Cre2, we examined prolactin levels, time to conception, litter size, pup weight and survival; and all were normal. We then examined the metabolic impact of selective GH elevation in the double KO model and found that, KO mice fed either a low-fat (LF), standard chow (CF), or high-fat (HF) diet starting at 4wks of age, were larger than diet-matched controls. The increase in weight was attributed to an increase in lean mass, while total fat mass did not differ within diet (by NMR). However, KO mice had less subcutaneous fat, with no change in visceral fat. Consistent with the well known anti-insulin effects of GH, ITT revealed insulin sensitivity was reduced in female KO mice, independent of diet, while only HF-fed male KO mice were insulin resistant, with elevated fed glucose levels. Despite reduced insulin sensitivity, LF- or CF-fed KO mice were more glucose tolerant (GTT) compared to controls, where these differences were reduced with HF-feeding. Improved glucose clearance in male CF-KO mice was associated with an increase in glucose-stimulated insulin release. These initial results suggest increases in endogenous GH levels improve glucose clearance through enhanced insulin output and/or increased lean mass when caloric intake is moderated, where elevated IGF-I may also contribute to this response. In contrast, when caloric intake is in excess, GH exacerbates insulin resistance and glucose homeostasis deteriorates. Surprisingly, elevated endogenous GH could not protect against fat accumulation but modifies fat distribution.

1 Romero CJ et al., Mol Endocrinol 2010; 24(5):1077-89
2 Luque RM et al., Endocrinology 2007; 148(5):1946-53

Sources of Research Support: Grants RYC-2007-00186, JC2008-00220, BFU2008-01136/BFI awarded to RML; FI06/00804 awarded to JCC; National Institute of Diabetes and Digestive and Kidney Diseases Grant 30677and Veterans Affairs Merit Award to RDK.

Nothing to Disclose: MDG, JC-C, CVA, QL, CRK, JCB, RML, RDK

Pub # P1-132

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Insulin-Like Growth Factor Signaling Regulates Stem/Progenitor Cells in Mammary Epithelium

Author String L Rota, DA Lazzarino, S Shushanov, D LeRoith, TL Wood
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Body The insulin-like growth factor (IGF) ligands, IGF-I and IGF-II, are potent mitogens and survival factors essential for embryonic growth and body size. In addition, the IGF signaling axis regulates normal postnatal growth and development of the mammary epithelium. The goal of this study was to determine if IGF signaling regulates epithelial stem and progenitor cell populations during mammary gland development. We analyzed primary mammary epithelial cells (pMECs) expressing a dominant-negative kinase-dead human IGF-1R transgene, under control of the MMTV promoter. In vivo loss of IGF signaling in the mammary epithelium resulted in a 50% reduction of cells within the CD24⁺CD29^{high}Sca-1⁻/lowLineage⁻ (Lin⁻), mammary stem- and committed myoepithelial-enriched pMEC fraction, as measured by flow cytometric analysis. In contrast, the CD24⁺CD29^{lo}CD61⁺Lin⁻ luminal progenitor epithelial population increased 2-fold in transgenic glands. We also observed a defect in ductal branching and alveolar budding of late virgin glands, a time that correlates with multiple estrus cycles and alveolar progenitor cell expansion. Using an in vitro mammosphere culture system to enrich mammary stem/progenitor cell populations, we further demonstrated that IGF-1R signaling is essential for growth of mammospheres. Either IGF-I or IGF-II was capable of recapitulating the number and size of primary spheres obtained in standard high insulin culture media. Moreover, addition of a blocking antibody to the IGF-1R significantly reduced sphere growth. Spheres cultured under conditions of IGF-1R stimulation contained bipotential progenitors as measured by the dual expression of myoepithelial and luminal lineage markers, cytokeratin 14/cytokeratin 18, respectively. These results suggest that both mammary epithelial progenitor cell expansion and stem cell renewal are regulated by IGFs. Taken together, the data support the hypothesis that IGF signaling is required for the maintenance and renewal of stem cells in the mammary gland.

Sources of Research Support: NIH DK60612 to TLW; NIH CA128799 to DL; New Jersey Commission on Cancer Research to LR; Ruth Estrin Goldberg Memorial for Cancer Research to DAL.

Nothing to Disclose: LR, DAL, SS, DL, TLW

Pub # P1-133

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Muscle Regeneration in Face of Reduced Circulating IGF-I

Author String A Vijayakumar, H Sun, S Yelamanchili, S Yakar
Mount Sinai School of Medicine, New York, NY

Body Insulin-like growth factor-1 (IGF-1) has well-documented roles in maintenance of muscle mass and function¹ as well as in skeletal muscle hypertrophy and regeneration². However it is yet unclear whether tissue or serum IGF-1 plays a predominant role in skeletal muscle regeneration. To address this, we utilized the ALSKO mouse model which has a 65% reduction in circulating IGF-1 levels due to the inability to form the IGF-1-IGFBP-ALS stabilizing ternary complex in serum³. Muscle injury was induced by local injection of cardiotoxin into the tibialis of 16 week old female control and ALSKO mice and the tissue was analyzed 1, 3, 7, 14 and 21 days after injury, which encompasses the time period over which the injured muscle completely heals. We found that ALSKO mice demonstrated reduced phosphorylation of the IGF-1 receptor (35%)/ Akt (81%)/ mTOR (58%) pathway during the course of the regeneration process. Interestingly, however, ALSKO mice demonstrated increased activation of the MAPK pathway, evident by increased Erk phosphorylation (94%). These data suggest that ALSKO mice manifest compensatory mechanism(s) to maintain muscle mass and repair muscle injury in face of significantly reduced circulating IGF-1 levels. Identifying the factors that mediate this compensatory response in the ALSKO mice would reveal new targets for treating muscle wasting disease.

1 Philippou A, Halapas A, Maridaki M, Koutsilieris M. Type I insulin-like growth factor receptor signaling in skeletal muscle regeneration and hypertrophy. *Journal of Musculoskeletal & Neuronal Interaction*. 2007 Jul-Sep; 7(3):208-18

2 Fernández AM, Kim JK, Yakar S, Dupont J, Hernandez-Sanchez C, Castle AL, Filmore J, Shulman GI, LeRoith D. *Genes and Development*. 2001 Aug; 15(15):1926-34

3 Yakar S, Rosen CJ, Beamer WG, Ackert-Bicknell CL, Wu Y, Liu JL, Ooi GT, Setser J, Frystyk J, Boisclair YR, LeRoith D. Circulating levels of IGF-1 directly regulate bone growth and density. *Journal of Clinical Investigation*. 2002 Sep; 110(6):771-81

Nothing to Disclose: AV, HS, SY, SY

Pub # P1-134

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Co-Recruitment of the NAPDH Oxidase Nox4 and Src to the Scaffolding Protein SHPS-1 Results in Localized Src Oxidation and Activation, Which Mediates Enhancement of the VSMC Proliferative Response to IGF-I

Author String G Xi, X Shen, DR Clemmons
University of North Carolina at Chapel Hill, Chapel Hill, NC

Body Hyperglycemia results in increase ROS generation in vascular cells and this is required for IGF-I-stimulated Src and MAP kinase activation, as well as increased VSMC proliferation. It has been proposed that Src oxidation is required for its activation. Since we have shown that Src must be activated for VSMC to proliferate in response to IGF-I and that it is activated while it is associated with SHPS-1, we investigated whether Nox4 (which is activated by hyperglycemia) is recruited to SHPS-1 and whether it generates ROS locally which oxidizes and activates Src in response to IGF-I. In order to disrupt Nox4/SHPS-1 association, we determined how Nox4 was recruited to the SHPS-1 complex. Protein sequence analysis identified a typical SH2 domain binding site, 491YVNI, in Nox4 and IGF-I stimulated an increase in Nox4 tyrosine phosphorylation. Since the direct binding of Nox4 to SHPS-1 was excluded, we postulated that Nox4 associates with SHPS-1 via a SH2 domain containing protein that associated with the SHPS-1 signaling complex. To support this hypothesis expression of a Nox4 Y491F substitution mutant was shown to eliminate IGF-I-stimulated Nox4/SHPS-1 association and Src oxidation and activation. Downstream signaling events, such as AKT and MAPK activation, were also impaired in Y491F overexpressing cells and they were enhanced by overexpression of wild type Nox4. Consequently, IGF-I-stimulated cell proliferation was impaired in Y491F overexpressing cells (e.g., 1.26 ± 0.11 fold vs. 2.06 ± 0.16 fold increase, $p < 0.05$) and enhanced in wild type Nox4 overexpressing cells (2.50 ± 0.12 fold vs. 2.06 ± 0.16 fold increase, $p < 0.05$) compared to LacZ control cells. To exclude the possibility that these changes were caused by the alteration of mitochondrial derived ROS, DIDS, a mitochondrial source superoxide blocker, was employed. The results clearly showed that blockage of mitochondrial derived superoxide did not change IGF-I-stimulated Src oxidation and activation or IGF-I-stimulated downstream signaling in VSMC exposed to hyperglycemia. Furthermore in vitro oxidation assays clearly indicated that Nox4 could mediate Src oxidation. We conclude that the effect of hyperglycemia on enhanced IGF-I signaling is not mediated through a generalized increase in ROS but instead by the specific oxidase, Nox4 that has to be recruited to SHPS-1, leading to local ROS generation which is essential for SHPS-1 associated Src oxidation and activation in response to IGF-I.

Nothing to Disclose: GX, XS, DRC

Pub # P1-135

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Insulin-Like Growth Factor Binding Protein-3 (IGFBP-3) Enhances Adherens Junctions and Reduces Inflammation by Reduction of Acid Sphingomyelinase (ASMase) to Maintain the Blood Retinal Barrier (BRB) after Injury

Author String MB Grant, LC Shaw, S Li Calzi, X Qi, JL Kielczewski, Q Ruan, J Cai, L Wu, RN Mames, SM Firth, R Baxter, ME Boulton, JV Busik
University of Florida, Gainesville, FL; University of Florida, Gainesville, FL; University of Sydney, St Leonards, Australia; , Gainesville, FL; Michigan State University, East Lansing, MI

Body **Purpose:** IGFBP-3 has well characterized vascular protective effects in both the micro- and macrovasculature; however, IGFBP-3 effect on retinal vascular permeability has not been investigated. In this study, we examined the effects of IGFBP-3 (non-IGF-1 binding mutant) in sustaining the blood retinal barrier (BRB) integrity using *in vitro* and mouse models of retinal barrier dysfunction and injury. **Methods:** BRB integrity was assessed *in vitro* by both transendothelial resistance (TER) and fluorescent flux following IGFBP-3 (100 ng/ml) treatment of bovine retinal endothelial cells (BREC). *In vivo*, the ability of IGFBP-3 to regulate the BRB was assessed using two distinct experimental mouse models; VEGF-induced retinal vascular permeability or laser induced retinal vessel obliteration. BRB permeability was quantified by intravenous injection of fluorescein isothiocyanate-bovine serum albumin (FITC-BSA) followed by digital confocal image analysis of retinal flatmounts. Claudin-5 and VE-cadherin localization at interendothelial junctions was confirmed by immunofluorescence in retinal flatmounts. Acid sphingomyelinase (ASMase) mRNA expression levels and activity were measured in mouse retinas following injury and IGFBP-3 treatment. **Results:** When IGFBP-3 was applied to the basolateral surface of BREC, TER increased and fluorescent flux decreased. The converse was true when IGFBP-3 was applied to the apical side of BREC. *In vivo*, intravitreal injection of IGFBP-3 reduced vascular leakage in VEGF injected mice and stabilized claudin-5 and VE-cadherin expression in junctional complexes, compared to VEGF alone injected mice. At four days post injury, in IGFBP-3 treated laser injured mouse retinas, vascular permeability was reduced compared to retinas of laser alone mice (p<0.05). In laser-injured retinas, IGFBP-3 decreased mRNA expression and activity levels of ASMase, the central enzyme of sphingolipid metabolism converting sphingomyelin to pro-inflammatory and pro-apoptotic ceramide, compared to laser alone mice (p<0.05). **Conclusions:** These data support that IGFBP-3 increases the integrity of retinal barrier both NIH grants EY007739, U01 HL087366, HL56912, and *in vitro* and *in vivo*. *In vivo*, IGFBP-3 increases the expression of stabilizes adherens junction proteins to reduce BRB permeability and this improvement is associated with a reduction in ASMase levels.

Sources of Research Support: NIH grants EY007739, U01 HL087366, HL56912, and HL102033.

Nothing to Disclose: MBG, LCS, SLC, XQ, JLK, QR, JC, LW, RNM, SMF, RB, MEB, JVB

Pub # P1-136

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Molecular and Functional Characterization of an IGF-II mRNA Binding Protein-(IGF2BP2b) Gene in Zebrafish

Author String M Li, X Rong, D Chen, L Lu, Y Li, C Duan
Ocean University of China, Qingdao, China; University of Michigan, Ann Arbor, MI

Body Insulin-like growth factor 2 mRNA binding protein-2 (IGF2BP2) belongs to the highly conserved VICKZ family of RNA-binding proteins. Proteins of this family recognize specific RNA targets and control their localization, stabilization, and translation. Recent genome-wide association studies have linked SNP in human IGF2BP2 intron 2 to type 2 diabetes. However, the biological actions of IGF2BP2 and its target RNAs are poorly understood. Here we identified and characterized an IGF2BP2 gene from zebrafish. Structural, phylogenetic, and comparative genomic analyses indicated that it is an ortholog of human IGF2BP2. Zebrafish *Igf2bp2* contains four KH domains but lacks any RRM domain. Zebrafish *igf2bp2* mRNA was ubiquitously expressed during zebrafish embryogenesis and in adult tissues. Overexpression of IGF2BP2 inhibited zebrafish embryos growth and development but had no effect on patterning. Targeted knockdown of zebrafish *igf2bp2* by antisense morpholino caused defects in many parts of the brain. It also resulted in smaller eyes and disrupted notochord in the tail region. No discernible effects were observed in muscle or vascular system. These results suggest that IGF2BP2 play an important role in embryogenesis and provide new information into the structure, gene expression, and in vivo function of IGF2BP2.

Sources of Research Support: NSF Research Grant IOB 0110864 and Natural Scientific Foundation of China Grant# 30928021.

Nothing to Disclose: ML, XR, DC, LL, YL, CD

Pub # P1-137

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Molecular Mechanisms and Functional Analysis of IGF-I and FGF-2 Cross-Talk in Neuroblastoma: A Role for miRNAs

Author String S Higgins, GA Werther, VC Russo
Murdoch Childrens Research Institute, Parkville, Australia; University of Melbourne, Parkville, Australia

Body
An early event in the pathogenesis of NB appears to be the arrested differentiation of neuroblasts. We recently demonstrated that FGF-2- induced growth arrest and differentiation of tumorigenic NB cell lines involves inhibition of IGF-I responses, activation of intracellular pathways (eg. MAPK), induction of cell cycle regulator p21, and differential regulation of target genes Id 1-3(1).
The best characterised genetic abnormality in NB is MYCN amplification. Recent studies have suggested that MYCN directly modulates a number of miRNAs in various cancers including NB. MiRNA dysregulation is commonly associated with tumorigenesis. We have therefore performed an miRNA expression profile to investigate which miRNAs are differentially modulated in response to IGF-1 (proliferation), FGF-2 or IGF-I/FGF-2 (both leading to differentiation) treated NB cells. We demonstrate, for the first time, that IGF-I specifically regulates (by >30%) a pool of 27 miRNAs (8[uarr] [e.g. Let7 family]; 19[darr][eg. mir21, mir200c]), while FGF-2 has opposing effects on 11 miRNAs from this pool (9[uarr] [e.g. mir21, mir200c]; 19 [darr][eg. Let7 family]). For 10 of the 27 miRNAs IGF-I maintains the same regulation in the presence of FGF-2, while for the remaining 17 miRNAs IGF-I regulation is reversed by FGF-2. Validation studies are in progress.
Neuroblastoma cells, of embryonic neural crest origin, possess a pool of cancer stem cells capable of unregulated self renewal, thus contributing to continual tumor growth. These cells express pluripotency genes including OCT4, NANOG, SOX15, KLF4, and TERT, all of which we now show are regulated in response to IGF-I, FGF-2 and IGF-I/FGF-2. Since miRNAs are known to regulate pluripotency genes, we are currently selecting NB cells according to expression of the neural crest stem cell marker CD133+, in order to determine their miRNA expression profile and thus their likely role in mediation of the IGF/FGF-2 regulation of pluripotency genes.
In conclusion, we show that arrested differentiation of neuroblasts is controlled by complex molecular mechanisms involving modulation of microRNA, pluripotency genes and possibly maintenance/expansion of the cancer stem cell pool. Target manipulation at various levels of this molecular network might be exploited to promote NB differentiation and ultimately assist with the treatment / management of this devastating paediatric cancer.

(1) Higgins S et al., Endocrinology 2009; 150:4044

Sources of Research Support: The National Health and Medical Research Council (NHMRC) of Australia Project Grant #1008062. SH is recipient of an NHMRC PhD Scholarship.

Nothing to Disclose: SH, GAW, VCR

Pub # P1-138

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title The Heparin Binding Domain of IGFBP-2 Increases Proliferation of Human Hematopoietic Stem Cells *In Vitro*

Author String AC Breggia, EM McMonagle, M Kawai, DR Clemmons, CJ Rosen
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Body Insulin Like Growth Factor Binding Protein-2 (IGFBP-2) is one of 6 binding proteins which regulate IGF signaling by promoting or inhibiting receptor binding. IGF independent effects of IGFBP-2 are thought to exist and are possibly mediated through the RGD or HBD (heparin binding) domains. We have demonstrated that a peptide analogous to the heparin binding domain (HBD) that is unique to IGFBP-2 increases bone density of metacarpal explants and rescues dysregulated hematopoiesis *in vivo* in the IGFBP-2 global knockout. As IGFBP-2 is reportedly a necessary factor for expansion of human hematopoietic stem cells *in vitro*, we investigated whether this effect could be mediated by the HBD (1). CD34+ human hematopoietic stem cells (HSC, Stem Cell Technologies) were cultured in the presence of HBD, a scrambled control peptide or received no treatment. At day 7, cell proliferation and viability was measured and cells analyzed by flow cytometry for each condition. Cell numbers were significantly increased for all conditions from day 0 to day 7 and viability was maintained at [ge]82%. While there was no significant difference in the no treatment and scrambled peptide controls, there was significantly more expansion of HSC treated with the HBD compared to these controls. Cell numbers increased from 0.7×10^4 cells/well at day 0 to 4.75×10^4 cells/well (no treatment), 2.92×10^4 cells/well (scrambled control peptide) and 7.92×10^4 cells/well (HBD) (N=3, p=0.002) Flow cytometric analysis demonstrated the majority of HSC treated with the HBD remained undifferentiated as [ge]72% of the cells were positive for the stem cell marker CD34. We have identified a putative receptor or human HSC to which the HBD binds. Future investigations will determine if this receptor-ligand interaction is responsible for HSC expansion *in vitro* and seek to identify the signaling mechanisms activated by HSC receptor-HBD ligand binding. Use of the HBD for HSC expansion prior to bone marrow transplantation could be an important therapeutic option to improve transplant outcomes.

(1) Zhang CC et al., Blood 2008;11:3415

Nothing to Disclose: ACB, EMM, MK, DRC, CJR

Pub # P1-139

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title GH and IGF-I Ameliorate Liver Steatosis and Fibrosis in Nonalcoholic Steatohepatitis Mouse Model

Author String H Nishizawa, G Iguchi, M Takahashi, M Yamamoto, K Suda, Y Okimura, H Kaji, K Chihara, Y Takahashi
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University of Hyogo, Awaji, Japan; Hyogo Prefectural Kakogawa Medical Center, Kakogawa, Japan

Body

(Background)
It has been suggested that nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are frequently observed in patients with adult growth hormone deficiency (AGHD). NASH is a serious disease because it progresses to liver cirrhosis and there is no established treatment for this disease. Insulin resistance, mitochondrial dysfunction, and aberrant activation of hepatic stellate cells are causally related with the pathogenesis of NASH. Recently we have reported that NASH accompanied with AGHD was dramatically ameliorated by GH replacement therapy (e.g. (1)). These results suggest that GH and/or IGF-I play an important role in liver and have a potential therapeutic application for NASH. The aim of this study is to apply GH or IGF-I for a treatment of general NASH and to clarify its molecular mechanisms.

(Method)
Methionine-choline-deficient diet-fed *db/db* (MCD *db/db*) mouse is one of established animal models for NASH. We examined the histological and metabolic effects of chronic GH or IGF-I administration in the physiological range in MCD *db/db* mice. We further analyzed the mechanisms by which GH or IGF-I prevents NASH development.

(Results)
IGF-I improved insulin sensitivity and glucose intolerance. Histologically, GH, especially IGF-I significantly ameliorated steatosis and fibrosis in the liver. In the aspects of molecular mechanisms, interestingly, IGF-I treatment increased mitochondrial volume and DNA content in hepatocytes. Recently it has been reported that cellular senescence of stellate cells inhibits fibrosis in the liver (e.g. (2)). Of note, we found that IGF-I induces cellular senescence in stellate cell line, LX2 cells and rat primary hepatic stellate cells, suggesting that IGF-I improved fibrosis via modulating the function of stellate cells by induction of cellular senescence.

(Conclusion)
These data indicate that GH and particularly IGF-I have a therapeutic potential for the treatment of general NASH. It is suggested that these effects were exerted by improving mitochondrial function in hepatocytes and by inhibiting stellate cell activation that links to fibrosis.

(1) Yutaka Takahashi et al., *Gastroenterology* 2007; 132:938
(2) Valery Krizhanovsky et. al., *Cell* 2008; 134:657

Nothing to Disclose: HN, GI, MT, MY, KS, YO, HK, KC, YT

Pub # P1-140

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Dissecting the IGF-I and FGF-2 Crosstalk in Neuronal Differentiation: Signaling Pathways and the Regulation of EWS/Fli1 and Downstream Target p21WAF1/Cip1

Author String MH Chao, DF Newgreen, GA Werther, VC Russo
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Body We have previously uncovered an intricate crosstalk between IGF-I and FGF-2 systems in restoring neuronal differentiation in neuroblastoma (NB) cells. Combined IGF-I/FGF-2 or FGF-2 treatment alone induced a differentiation response, while IGF-I treatment alone induced proliferation. Thus the apparent opposing effects of IGF-I (proliferation) and FGF-2 (differentiation) were integrated to produce a cell differentiation signal. This suggested that the pro-tumorigenic actions of IGF-I in NB cells were altered by FGF-2, such that the cellular response to IGF-I was switched from proliferation to differentiation. We therefore sought to identify key determinants of differentiation control pathways (ie. molecular switches of IGF-I action) by investigating the IGF-I/FGF-2 crosstalk in NB cell line SK-N-MC.

Signalling pathway analysis revealed IGF-I/FGF-2 and FGF-2 treatment alone induced rapid and sustained activation of the Erk1/2 MAPK pathway. Using phospho-kinase array analysis the MAPK pathway kinases, MSK1/2 and p38 α MAPK and transcription factors, CREB and c-Jun were similarly up-regulated by IGF-I/FGF-2 and FGF-2 treatments. The differential regulation of GSK-3 β by IGF-I (inactivation) and FGF-2 (activation) suggested that this molecule was a potential molecular switch for IGF-I signalling. However, GSK-3 β inhibitors (TDZD and LiCl) and siRNA revealed FGF-2-induced activation of GSK-3 β was not involved.

Another putative candidate was EWS/Fli1, an oncogenic fusion protein promoting tumorigenesis of SK-N-MC cells. EWS/Fli1 also acts as an aberrant transcription factor for oncogenes such as c-Myc, Id2 and IGF-I and represses those implicated in growth arrest and differentiation (i.e. p21). The expression of the EWS/Fli1 protein was maintained in the presence of IGF-I while it was down-regulated by IGF/FGF-2 and FGF-2 treatment alone. We were also able to demonstrate that mRNA and protein levels of cyclin dependent kinase inhibitor p21 WAF1/Cip1, was dramatically induced by IGF-I/FGF-2 or FGF-2 alone, an effect reversed by the FGF receptor inhibitor, PD173074.

Further experiments designed to over-express, knock-down (using RNAi) or block (small molecules; pathway inhibitors) elements of the IGF-I and FGF-2 signalling pathways including EWS/Fli1 and p21WAF1/Cip1 are planned. The ability to selectively modulate these pathways points to alternative novel targets and therapeutic molecules for the management and treatment of NB.

Sources of Research Support: The National Health and Medical Research Council (NHMRC) of Australia Project Grant # 1008062.

Nothing to Disclose: MHC, DFN, GAW, VCR

Pub # P1-141

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Heterozygous *Growth Hormone Receptor (GHR) Variant, c.899dupC*: A Novel Dominant Negative *GHR* Mutation Associated with GH Insensitivity and IGF-I Deficiency

Author String MA Derr, J Aisenberg, P Fang, RG Rosenfeld, V Hwa
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Body The human growth hormone receptor (GHR), upon binding GH, induces JAK2-mediated phosphorylation of GHR intracellular tyrosines, which then recruit components of multiple signaling pathways, including STAT5b, with subsequent regulation of the growth promoting insulin-like growth factor (IGF)-1. Mutations found in the *GHR* gene are the most common cause of growth hormone insensitivity (GHI), a condition characterized by pronounced IGF-I deficiency (IGFD) and severe short stature. We had previously identified a novel compound heterozygous *GHR* mutation in a patient presenting with GHI (1). One of the heterozygous mutations was R211H (mature GHR), a variant lacking adequate functional studies but associated with GHI (2). The second heterozygous mutation was a novel duplication of a nucleotide in exon 9 (*c.899dupC*), within the critical JAK2-binding Box 1 region of the GHR intracellular domain; the duplication predicted a frameshift and early protein termination.

Objective: Reconstitution studies (in HEK293 cells) were employed to evaluate the individual and synergistic effect(s) of hGHR:R211H and hGHR:*c.899dupC*, compared to wild-type hGHR, on GH-induced STAT5b signaling and transcriptional activities.

Results: The hGHR:R211H variant was readily expressed and, unexpectedly, GH-induced STAT5b phosphorylation, was comparable to that induced by wild-type hGHR. The truncated, immuno-detected hGHR:*c.899DupC* variant, in contrast, was unresponsive to GH, consistent with the variant lacking the JAK2-binding Box 1 region. To mimic a compound heterozygous state, hGHR:R211H and hGHR:*c.899dupC* were co-expressed, and, strikingly, presence of hGHR:*c.899dupC* effectively abolished the GH-induced STAT5b activities that were observed with hGHR:R211H alone. Further, hGHR:*c.899DupC* dose-dependently reduced the GH-induced STAT5b activities associated with hGHR:R211H. This dominant-negative effect was also observed when hGHR:*c.899DupC* was co-expressed with wild-type hGHR.

Conclusion: Two important insights were gained from our study: first, the R211H variant, contrary to an earlier report (2), appeared to function like wild-type GHR and, therefore, unlikely to cause GHI. Second, the *c.899DupC* variant is a novel dominant negative mutation which disrupts normal GHR signaling, and is the cause for the GHI phenotype of the reported patient (1). Our study emphasize the importance of performing functional studies when correlating genotype with phenotype in disease states.

(1) Aisenberg J et al., *Horm Res Paediatr* 2010; 74:406

(2) Goddard AD et al., *N Engl J Med* 1995; 333:1093

Sources of Research Support: The March of Dimes Foundation (RGR).

Nothing to Disclose: MAD, JA, PF, RGR, VH

Pub # P1-142

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title miR-19a and mi-R125b Are Induced and the Growth-Suppressing IGFBP-3/IGFBP-3R System Is Suppressed during Progression of Prostate Cancer

Author String R Gandee, L Mohanraj, Y Oh
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Body Micro-RNAs (miRNAs) are 22 nucleotide long RNA molecules that are known to regulate the translation and expression of proteins within a cell. Binding of the miRNAs to a nucleotide sequence in the 3'-UTR of mRNA can inhibit specific proteins through translational inhibition or degradation of the mRNA precursor of that protein. Recent studies have demonstrated that dysregulation of specific miRNAs is attributed to the oncogenic properties of cancer cells.

IGFBP-3 is known to be a potent tumor suppressor in a variety of cancers. Recently, our lab has identified a specific receptor, IGFBP-3R that interacts with IGFBP-3 to promote anti-tumor activity. We further demonstrated that the IGFBP-3/IGFBP-3R axis is impaired in breast and prostate cancer. Using Targetscan, miR-19a and miR-125b were identified as possible miRNAs that could affect the expression of IGFBP-3 and IGFBP-3R, respectively. To investigate the impact of miRNA-19a and miR-125b on the tumor-suppressing IGFBP-3/IGFBP-3R system in prostate cancer, we investigated two distinct in vitro prostate tumor progression systems: P69-M12 and BPH1-CAFTD-02 cell systems.

In our study, we observed reduced levels of IGFBP-3 and IGFBP-3R in CAFTD-02 and M12 (cancerous prostate cells) when compared to BPH1 and P69 cells (non-cancerous prostate cells), respectively. Furthermore, M12 and CAFTD-02 cells with reduced levels of IGFBP-3 and IGFBP-3R, also possess elevated endogenous levels of miR-19a and miR-125b. This suggests that a correlation exists between the levels of miR-19a and IGFBP-3 and miR125b and IGFBP-3R respectively suggesting that these miRNAs may regulate the expression of IGFBP-3 and IGFBP-3R. Transfection of P69 and BPH-1 with pre-miR-19a and pre-miR-125b showed reduction in the levels of IGFBP-3 and IGFBP-3R respectively, whereas transfection with anti-miR-19a and anti-miR-125b in M12 and CAFTD-02 cell lines showed an increase in the levels of IGFBP-3 and IGFBP-3R respectively.

Further investigation of miR19a and miR125b on the IGFBP-3/IGFBP-3R system will enable us to better understand and utilize miRNA as a diagnostic tool, leading to the development of specific therapies designed to reduce the aberrant levels of miRNAs in cancer cells, thus restoring the appropriate levels of IGFBP-3 and IGFBP-3R, specifically. This will restore the capacity of the affected cells to undergo apoptosis and inhibit further cellular growth and proliferation.

Nothing to Disclose: RG, LM, YO

Pub # P1-143
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)
Title Development of Tamoxifen Resistance in MCF7 Breast Cancer Cells Is Associated with Greater Dependence upon IGFBP-2 for Cell Survival
Author String EJ Foulstone, JMP Holly, CM Perks
University of Bristol, Bristol, UK

Body
Background
The majority of newly diagnosed breast cancer is hormone dependent with first line treatment being anti-oestrogen therapy, typically tamoxifen. Unfortunately acquired resistance to tamoxifen and other anti-oestrogens is a major clinical problem. Increased expression of IGFBP-2 has been linked to breast cancer progression and upregulation of IGFBP-2 has been seen in anti-oestrogen resistant breast cancer cell lines indicating that this protein may be involved in resistance.

Aim

We therefore investigated whether IGFBP-2 was important for cell growth and survival in MCF7 cells resistant to tamoxifen in comparison to parental cells.

Methods

Tamoxifen resistant (Tam-R) and parental MCF7 (pMCF7) cells were kindly supplied by Julia Gee (Cardiff University). Low passage MCF7 cells were from ATCC. siRNA technology was used to knockdown IGFBP-2, with proliferation and death determined by cell counting with trypan blue. Proliferation in response to IGF-II was investigated using ³[H]- thymidine uptake.

Results

Tam-R cells expressed more IGFBP-2 than pMCF7 cells and while the amount of ER- α was lower it was still present. Knockdown of IGFBP-2 using 1nM siRNA was equivalent in all cells and reduced the number of live Tam-R cells by 28% (n=3 p<0.05) suggesting that these cells need IGFBP-2 for proliferation. However, the decrease in live cell number was equivalent to that in pMCF7 cells indicating resistance has not altered the requirement of IGFBP-2 for proliferation in these cells. In contrast, cell death in the Tam-R cells induced by IGFBP-2 loss was far greater than in the pMCF7 or low passage MCF7 cells, 52% (n=3 p<0.05) compared to 11% and 19% respectively. We have shown previously that binding of secreted IGFBP-2 by high dose IGF-II blocked IGFBP-2 induced PTEN reduction, reducing proliferation in MCF7 cells (1). Tam-R cells also showed decreased proliferation at high doses of IGF-II indicating sequestration of IGFBP-2 and possible modulation of PTEN levels.

Conclusion

These data show that Tam-R cells utilise IGFBP-2 in a similar way to pMCF7 cells to increase proliferation and modulate PTEN levels, however resistance to tamoxifen was associated with an enhanced dependence on IGFBP-2 as a survival factor. Understanding how tamoxifen resistant cells can be targeted for treatment will be important for future therapy regimes. IGFBP-2 appears a legitimate place to start.

1. Perks C et al., *Oncogene* 2007; 26:5966

Sources of Research Support: Breast Cancer Campaign project grant.

Nothing to Disclose: EJF, JMPH, CMP

Pub # P1-144

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Dissecting the Mechanisms of IGFBP-2 Nuclear Translocation in Neuroblastoma Cells

Author String WJ Azar, GA Werther, VC Russo
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Body Disruption in the balance of the IGF system components leading to excessive proliferation, motility and survival signals have been implicated in the development of many different tumor types. IGFBP-2, expressed in both serum and in tumor tissues and with levels in direct proportion to tumor aggressiveness, is one of the most significant genes in the signature of major aggressive cancers. We have previously shown that IGFBP-2 enhances proliferation and invasion of neuroblastoma (NB) cells, suggesting that IGFBP-2 activates a pro-tumorigenic gene expression program in these cells. Accordingly, our follow up gene expression profile analysis showed that IGFBP-2 over-expression results in the enhancement of genes involved in proliferation/survival, migration/adhesion and angiogenesis, including the up-regulation of vascular endothelial growth-factor (VEGF) mRNA. We now demonstrate up-regulation of VEGF mRNA expression and specific transcriptional activation of the VEGF luciferase promoter construct by IGFBP-2, an event that only occurs in the presence of intracellular IGFBP-2. We further show that nuclear entry of IGFBP-2 is required for activation of VEGF gene expression. Although no nuclear-localization signal (NLS) sequence has been described for IGFBP-2, an NLS-like consensus sequence was identified at 179PKKLRPP185. Site-direct mutagenesis of this putative NLS motif (179PNNLAPP185), resulted in cytoplasmic accumulation of the NLSmut-IGFBP-2, while wild type IGFBP-2 was actively transported into the nuclear compartment of digitonin treated cells. Cell fractionation of NB cells over-expressing wild type IGFBP-2 or NLSmut-IGFBP-2 showed abundant cytoplasmic IGFBP-2, with nuclear IGFBP-2 only detected in the NB cells over-expressing wild type IGFBP-2. Similarly, over-expression of NLSmut-IGFBP-2 did not affect the VEGF transcriptional activation as seen in the presence of wild type IGFBP-2. Taken together, these results suggest that IGFBP-2 is translocated into the nucleus, an event requiring an intact NLS motif. This leads, directly or indirectly, to functional consequences including activation of the VEGF promoter.

Sources of Research Support: National Health and Medical Research Council (NHMRC) of Australia Project Grant # 1008062.

Nothing to Disclose: WJA, GAW, VCR

Pub # P1-145

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title The Alzheimer Disease Risk Gene Clusterin Is Expressed in the Developing Choroid Plexus under the Regulation of Notch but Not IGF Signaling

Author String S Jiao, W Dai, V Korzh, C Duan
University of Michigan, Ann Arbor, MI; Ocean University of China, Qingdao, China; National University of Singapore, Singapore

Body Recent genome-wide association studies have implicated the clusterin gene in the etiology of Alzheimer's disease. The expression and function of clusterin in the developing brain, however, is poorly understood. In this study, we have characterized the zebrafish clusterin gene and determined its structural conservation, developmental expression, and physiological regulation. The structure of the zebrafish clusterin gene and protein is similar to its human orthologue. Biochemical assays show that zebrafish Clusterin is a secreted protein that cannot bind IGFs. In adult zebrafish, clusterin mRNA is detected in many tissues. In early development, clusterin mRNA becomes detectable at 12 hpf and its levels gradually increase thereafter. In situ hybridization analysis indicates that clusterin mRNA is specifically expressed in the developing diencephalic and myelencephalic choroid plexus. Among various stresses tested, heat shock, but not hypoxic or ionic stresses, increases the levels of clusterin mRNA. Inhibition of the IGF-1 receptor-mediated signaling or overexpression of IGF ligands did not change clusterin mRNA levels. In comparison, inhibition or targeted knockdown of Notch signaling significantly increased clusterin mRNA expression in choroid plexus. These results suggest that clusterin is a marker of choroid plexus in zebrafish and its expression in the developing choroid plexus is under the regulation of Notch but not IGF signaling.

Sources of Research Support: NSF Research Grant IOB 0110864 and Natural Scientific Foundation of China Grant# 30928021. SJ is supported in part by a fellowship from China Scholarship Council. VK is supported by the A-STAR of Singapore grant for IMCB.

Nothing to Disclose: SJ, WD, VK, CD

Pub # P1-146

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Preliminary Characterization of a Transgenic Mouse with Selective *Igf2* Depletion in the Thymic Epithelium

Author String M Mottet, H Martens, O Dardenne, V Geenen
University of Liege, Liege-Sart Tilman, Belgium

Body

Background. During evolution, the thymus appeared almost concomitantly with the recombinase-dependent adaptative immune response to preserve potential autotoxicity inherent to this novel form of immunity. The thymus establishes central immunological self-tolerance by deletion of self-reactive T-cell clones (*recessive* self-tolerance) and generation of self-antigen specific natural regulatory T cells (*dominant* self-tolerance). During fetal ontogeny, all the genes of the insulin family are transcribed in the thymus according to a precise hierarchy: *Igf2* > *Igf1* > *Ins2* > *Ins1* (1). *Igf2* transcription is defective in the thymus of Bio-Breeding rats, an animal model of human type 1 diabetes (2), and *Igf2*^{-/-} mice display a lower tolerance to insulin suggesting that thymic IGF-2 could promote significant cross-tolerance to insulin (3).

Objective. This study aims at further determining the precise role of thymic IGF-2 in the establishment of central self-tolerance to the insulin family and to insulin-secreting beta cells in the pancreatic islets of Langerhans.

Methods. Because of the ubiquitous *Igf2* expression, the selective deletion of this gene in thymic epithelial cells (TEC) should provide important answers to this question. Since FOXN1 is the specific transcription factor for TEC, *Igf2-loxP* and *Foxn1-Cre* mice were crossed in our laboratory.

Results. The presence of the Cre transgene in transgenic TEC and its absence in control TEC was checked by PCR. The weight of transgenic thymus was significantly higher than wild type (WT) thymus, but the general thymic cytoarchitecture was not modified. After selective isolation of TEC, *Igf2* transcripts were almost completely depleted in transgenic TEC, and this was confirmed by *in situ* hybridization with *Igf2* probes. After 4 weeks, mean glycaemia was significantly higher in *Igf2*^{-/-Thy} (143 mg%) than in WT mice (112 mg%, N=20, P<0.005).

Conclusion. These preliminary data confirm a severe and selective *Igf2* depletion in TEC of transgenic mice. Blood glycaemia, pancreas histopathology, and immunological tolerance to insulin-epitopes will be further scrutinized both in *Igf2*^{-/-Thy} and WT mice.

1. Geenen V et al., Curr Opin Pharmacol 2010; 10:461
2. Kecha-Kamoun O et al., Diabetes Metab Res Rev 2001; 17:146
3. Hansenne I et al., J Immunol 2006; 176:4651

Sources of Research Support: Fund of Scientific Research (Belgium); Walloon Region of Belgium (Waleo 2 project Tolediab).

Nothing to Disclose: MM, HM, OD, VG

Pub # P1-147

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Epigenetic Regulation of IGFBP-5 and Estrogen Receptor (ER) in Mammary Glands and Tumors of Fetal Alcohol-Exposed Offspring

Author String C Crismale-Gann, TA Polanco, WS Cohick
Rutgers University, New Brunswick, NJ

Body Exposure to alcohol during fetal development increases susceptibility to carcinogen-induced mammary tumor in rats. We previously reported that tumors from alcohol-exposed offspring had decreased IGFBP-5 expression relative to tumors from control animals and that more tumors were ER negative. In the present study, we examined IGFBP-5 expression in early mammary gland development and investigated whether changes in IGFBP-5 expression and ER status could be due to epigenetic modifications. Pregnant Sprague Dawley rats were fed a liquid diet containing 6.7% ethanol, pair-fed an isocaloric liquid diet, or fed rat chow ad libitum from days 11 to 21 of gestation. In study 1, mammary tumors were induced with NMU at 50 days of age. Offspring were sacrificed at 23 weeks after NMU injection and tumors were collected. In study 2, offspring were euthanized at postnatal day (PND) 20, 40, or 80, and mammary glands were collected. Mammary expression of IGFBP-5 was decreased in fetal alcohol-exposed offspring at PND 40 relative to controls. Bisulfite DNA sequencing and methylation specific PCR were used to determine the methylation status of IGFBP-5 and ER in mammary glands and tumors of fetal alcohol-exposed rats from these two studies. Genomic DNA was isolated, digested with RsaI, and treated with sodium bisulfite. For bisulfite DNA sequencing, the only CpG island within the IGFBP-5 promoter and the first CpG island within the coding region were amplified using bisulfite specific primers. PCR products were then cloned using the pGEM-T Easy Vector and sequenced. For methylation specific PCR, bisulfite-treated DNA was amplified using two sets of primers that distinguish between methylated and unmethylated DNA. These primers were designed for the first CpG island within the IGFBP-5 coding region as well as for a CpG island spanning the ER promoter/exon I. Methylation of IGFBP5 and ER CpG islands was observed in both mammary glands and tumors. DNA methyltransferase 1 protein expression was also analyzed in mammary glands and tumors of fetal alcohol exposed animals and alterations in expression were found compared with control groups. Our data suggest a role for epigenetics in the increased susceptibility of carcinogen-induced mammary tumors in offspring exposed to alcohol in the fetal environment.

Nothing to Disclose: CC-G, TAP, WSC

Pub # P1-148

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title A New Automated Chemiluminescence Immunoassay System to Measure Insulin-Like Growth Factor I (IGF-I): Validation and Establishment of Reference Values

Author String M Bidlingmaier, J Manolopoulou, M Bougoussa, A Doering, RT Emeny, B Obermayer-Pietsch, J Spranger, AH Pfeiffer, J Kratzsch, AM Arafat
Ludwig-Maximilians-University, Medizinische Klinik Innenstadt, Munich, Germany; Immunodiagnostic Systems, Boldon, UK; Immunodiagnostic Systems, Liege, Belgium; Helmholtz Zentrum Muenchen - German Research Center for Environmental Health (GmbH), Neuherberg, Germany; Universit[au]tsklinik für Innere Medizin, Medizinische Universit[au]t Graz, Graz, Austria; Charité-University Medicine Berlin, Campus Benjamin Franklin, Berlin, Germany; University of Leipzig, Leipzig, Germany

Body Measurement of circulating IGF-I is widely used to diagnose GH-related disorders and monitor treatment efficacy. However, technical issues have been shown to reduce comparability of assay results across laboratories. Use of a common reference preparation as well as the availability of method specific normative data established from sufficiently large cohorts are important to allow meaningful interpretation of IGF-I data
Methods: A new automated chemiluminescence assay (iSYS IGF-I, IDS, Boldon, UK) employing monoclonal antibodies against IGF-I and including IGF-II displacement was validated according to CLSI guidelines. Method specific reference ranges were established by analysing samples from 2583 children (age 0 -18; 1067 males/1516 females) and 6671 adults (age 19 -95; 2799 males / 3872 females). Participants came from European and US based population studies (including KORA, KORA Age, MeSy BePo cohort Berlin, and Caliper) and did not have chronic kidney disease, liver disease, type I diabetes, cancer or pituitary diseases. IGF-I values were explored using several statistical models, and reference ranges were constructed using the LMS method (Cole TJ, 1990).
Results: The assay has a dynamic range of 10-1200ng/ml, analytical and functional sensitivities at 4.4ng/ml and 8.8ng/ml respectively, within- and between-assay CVs (1.4-7.2%), excellent linearity and recovery (99 to 108%). Calibration is against the new WHO standard (02/254), a defined mass content recombinant material. The assay does not cross-react (<0.01%) with insulin, pro-insulin or IGF-II and shows no interference from the 6 high affinity IGF-BPs.
As expected, IGF-I concentrations in the healthy population exhibit strong age dependency. Values increase until puberty followed by a steep decline until age 25, with a slower decrease thereafter. Peak IGF-I occurs earlier and is slightly higher in females. Later in life, IGF-I is slightly but significantly higher in males compared to females. Age- and sex-specific 2.5th and 97.5th centiles were derived from statistical modelling and an algorithm to calculate IGF-I SD scores for any given age is presented.
Conclusion: The IDS-iSYS IGF-I assay allows reliable quantification of circulating IGF-I concentrations without interference from binding proteins. Together with the age- and sex specific reference values established from an extremely large cohort of healthy subjects the assay presents a useful tool to assess IGF-I values across the human lifespan.

Cole TJ, Eur J Clin Nutr. 1990;44(1):45-60

Disclosures: MB: Consultant, Immunodiagnostic Systems Ltd. JM: Employee, Immunodiagnostic Systems Ltd. MB: Employee, Immunodiagnostic Systems Ltd. Nothing to Disclose: AD, RTE, BO-P, JS, AFHP, JK, AMA

Pub # P1-149

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Insulin-Like Growth Factor-I (IGF-I) Stimulates Interleukin-7 (IL-7) Synthesis and Secretion in Primary Cultures of Human Thymic Epithelial Cells

Author String L Goffinet, H Martens, V Geenen
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Body **Background.** The thymus is responsible for thymopoiesis, *i.e.* the generation of a diverse and self-tolerant T-cell repertoire, including self-antigen specific natural regulatory T cells. Two parameters of thymopoiesis, thymic output of naive T cells (estimated by sjTREC frequency) and intrathymic proliferation of T-cell precursors (estimated by sj/DB TREC ratio), are severely affected in adult patients with growth hormone (GH) deficiency (AGHD) and GH treatment is able to restore these parameters (1). In AGHD patients, sjTREC frequency is highly correlated to plasma concentrations of insulin-like growth factor (IGF-1), which mediates most of GH actions.

Hypothesis. We hypothesized that the thymotropic properties of the somatotrope GH/IGF-1 axis could be relayed by thymic interleukin 7 (IL-7), a cytokine that is essential for V(D)J recombination at the TCR locus and that modulates chromatin accessibility for recombinase-mediated generation of T-cell diversity.

Results. Primary cultures of human thymic epithelial cells (TECs) were treated with several doses of recombinant human IGF-1. Supernatants were then recovered and IL-7 concentrations were measured by a highly sensitive ELISA. IL-7 secretion by TECs was significantly increased by IGF-1 in a dose-dependent manner. In addition, the quantity of IL7 transcripts measured by RT-qPCR in TEC mRNAs was also stimulated by IGF-1. Specificity of these effects was assessed using the IGF-type 1 receptor (IGF-1R) -blocking antibody α IR3. The addition of α IR3 significantly decreased the IL-7 secretion by human TECs stimulated by IGF-1, thus demonstrating the direct involvement of IGF-1R.

Conclusions. These data show that, in humans, thymic IL-7 may be implicated in the mediation of the thymotropic properties of the somatotrope GH/IGF-1 axis. Through IL-7, the somatotrope axis might be implicated in (re)generation of TCR diversity.

(1) Morrhaye G et al., PLoS One 2009; 4:e5668.

Sources of Research Support: Fund of Scientific Research of Belgium; Pfizer Independent Research Grant.

Nothing to Disclose: LG, HM, VG

Pub # P1-150

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Transcription-Independent Overexpression of Insulin-Like Growth Factor II in Adrenal Hyperplasia and Cortisol-Producing Adenomas

Author String KS Nadella, C Johnson, E Patterson, M Almeida, L Isaac, S Koliavasilis, E Kebebew, K Pfeifer, C Stratakis
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Body Insulin like growth factor-II (IGF2), a key regulator of cell growth and development is tightly regulated in its expression during embryogenesis and normal growth through imprinting. H19 transcript, a noncoding RNA regulates the expression of IGF2. H19 and IGF2 are reciprocally imprinted genes, where H19 is expressed from paternal allele and IGF2 from maternal alleles. Biallelic expression of IGF2 resulting from loss of imprinting was reported in many cancers including adrenocortical tumors. Since alterations of the IGF2 axis are associated with adrenal tumors, we investigated the expression of IGF2 in three different subsets of human adrenal lesions; Massive Macro nodular Adrenocortical Disease (MMAD), Cortisol Producing Adenomas (CPA) and Primary Pigmented Nodular Adrenocortical disease (PPNAD) with mutations in *PRKARIA*. The aim of the study was to determine if IGF2 is differentially expressed between adrenal hyperplasia's and adenomas and to determine the utility of IGF2 as a biomarker and a possible therapeutic target. Western blotting analysis from 23 tumor samples revealed over expression of IGF2 protein in 4/6 MMAD, 4/6 CPA and 4/11 PPNAD lesions. Immunohistochemistry further confirmed the tumor specific accumulation of IGF2 protein in the cytoplasm. Interestingly, adrenals from *Prkar1a* knockout mice with altered PKA signaling showed marked up regulation of IGF2 compared to the wild type mice. To investigate the molecular mechanisms responsible for IGF2 over expression we screened the samples for loss of imprinting by measuring biallelic expression of IGF2 and H19 transcript through previously reported single nucleotide polymorphisms in IGF2 and H19 genes, respectively. We also quantitated total IGF2 mRNA using qRT-PCR. We did see loss of imprinting in some samples. However, no correlation was observed between loss of imprinting and mRNA expression levels and more importantly between IGF2 RNA and protein expression levels. Immunoblot analysis of IGFBP2, revealed its upregulation in some samples which didn't correlate with IGF2 levels. Furthermore sequencing of IGF2 revealed no sequence changes. Our data therefore suggest a post transcriptional mechanism for IGF2 protein stabilization that might have lead to the observed accumulation of IGF2 and therefore its availability for enhanced proliferation of adrenal tumor cells. Experiments are on to understand the functional relevance of increased IGF2 in these tumors.

Nothing to Disclose: KSN, CJ, EP, MA, LI, SK, EK, KP, CS

Pub # P1-151

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Statin Inhibits Radiation-Induced Fibrosis through Regulation of CTGF/IGFBP-rP2 Action in Lung Fibroblasts

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Body Connective tissue growth factor (CTGF)/Insulin-like growth factor binding protein-related protein 2 (IGFBP-rP2) is a potent regulator of fibroblast proliferation, cell adhesion, and stimulates extracellular matrix production. CTGF/IGFBP-rP2 mediates the fibrotic effects of TGF- β . TGF- β plays an important role in radiotherapy-induced fibrosis (RTIF). However, treatment with the TGF- β neutralizing antibody does not completely prevent RTIF in animal model, suggesting that CTGF/IGFBP-rP2 plays a pivotal role in fibrosis in a TGF- β -dependent and -independent manner. Furthermore, statins have been shown to inhibit fibrosis, but the specific mechanism remains to be elucidated.

To investigate the impact of CTGF/IGFBP-rP2 on RTIF, *in vivo* study was performed using mouse model. Immunohistochemical analysis revealed that trichrome staining was observed in the lungs of irradiated animals 14 weeks after irradiation (IR) indicating IR-induced fibrosis. Concomitantly, CTGF/IGFBP-rP2 was also detected 14-week post-IR. In agreement with *in vivo* results, CTGF/IGFBP-rP2 as well as fibrotic proteins was induced by TGF- β (0-25 ng/ml) and IR (0-10 Gy) dose dependently at mRNA and protein levels in normal human fetal lung fibroblasts. Furthermore, treatment with the TGF- β neutralizing antibodies resulted in complete inhibition of TGF- β -induced CTGF/IGFBP-rP2 and fibrotic protein production, but failed to inhibit their production induced by IR completely. These findings strongly suggest that IR may induce fibrosis through increased expression of CTGF/IGFBP-rP2 in a TGF- β -dependent and -independent manner. Interestingly, statins not only inhibited IR-induced CTGF/IGFBP-rP2 production as well as fibrotic proteins such as fibronectin and Collagen IV completely but also inhibited CTGF/IGFBP-rP2-induced fibrosis in the absence of TGF- β . These data strongly suggest that statins may inhibit RTIF at multiple levels including inhibition of TGF- β signaling pathway involved in CTGF/IGFBP-rP2 production and CTGF/IGFBP-rP2 signaling pathway attributing to enhanced expression of fibrotic factors.

Taken together, our findings strongly suggest that radiation-induced CTGF/IGFBP-rP2 plays a major role in the development of lung fibrosis by inducing fibrotic proteins through TGF- β -dependent and -independent manner. Further understating of the mechanisms of CTGF/IGFBP-rP2 action in RTIF may provide a new intervention strategy for prevention and treatment of fibrosis after RT.

Nothing to Disclose: AH, WL, CL, MI, MSA, YO

Pub # P1-152

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title A SNP in the Promoter Region of the IGFBP-5 Gene Is Associated with Circulating Levels of IGFBP-2

Author String RP Narayanan, RH Stephens, SG Anderson, KW Siddals, RL Oliver, JE Hudson, AH Heald, JP New, WER Ollier, JM Gibson
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Body A decrease in Insulin like growth factor binding protein -2 circulating levels is known to be associated with the metabolic syndrome(1).The IGFBP2 and IGFBP5 genes are syntenic on chromosome 2q33-34 and 2q33-36 respectively- about 20-40 kb apart. We studied the associations of single nucleotide polymorphisms (SNPs) in these two genes and circulating levels of IGFBP-2 in 459 patients with type 2 diabetes drawn from a Caucasian population in North West England. 18 SNPs were identified from the HapMap database (International HapMap project)and the subject population genotyped using the Sequenom iPLEX platform.IGFBP-2 levels were measured in the subject population using the RayBio ELISA commercial kit.Inter-assay coefficients of variation were up to 12% and intra-assay variation up to 10%. Linear regression analysis was carried out using Stata 10SE.We identified that the homozygous minor allele of SNP rs1978346 (A/G) in the promoter region of the IGFBP5 gene was associated with decreased circulating levels of IGFBP-2 (p 0.005, CI -0.46 to -0.08).This suggested possible functional coupling of the IGFBP2 and IGFBP5 genes at the 2q locus, and may have implications in the development and progression of co-morbidities of type 2 diabetes.

(1) Heald, A.H. et al. Insulin-like growth factor binding protein-2 (IGFBP-2) is a marker for the metabolic syndrome. *Exp Clin Endocrinol Diabetes* 114, 371-6 (2006).

Nothing to Disclose: RPN, RHS, SGA, KWS, RLO, JEH, AHH, JPN, WERO, JMG

Pub # P1-153

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title The Response of IGF-I and Pro-Collagen Type III Amino-Terminal Propeptide to the Administration of Recombinant Human IGF-I/IGF Binding Protein-3 in Recreational Athletes

Author String N Guha, I Erotokritou-Mulligan, SP Nevitt, M Francis, C Bartlett, D Cowan, EE Bassett, PH Sonksen, RIG Holt
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Body Background: Growth hormone is abused by athletes for its anabolic and lipolytic properties and there are anecdotal reports that insulin-like growth factor-I (IGF-I) is being abused as an alternative or additional doping agent. There is currently no test to detect IGF-I abuse. We propose that it will be possible to detect exogenous IGF-I administration by measuring serum biomarkers of the IGF/IGF binding protein (IGFBP) axis and markers of bone and collagen turnover.

Methods: The study received approval from the local ethics committee. We performed a randomised, double-blind, placebo-controlled study of 4 weeks treatment followed by 8 weeks washout. 56 recreational athletes (age 18-30 years, 30 men, 26 women) were randomly assigned to receive placebo, low dose (30 mg/day) recombinant human IGF-I (rhIGF-I)/rhIGFBP-3 complex or high dose (60 mg/day) rhIGF-I/rhIGFBP-3 complex. Treatment was self-administered by subcutaneous injection for 28 consecutive days. Serum IGF-I and the collagen marker, pro-collagen type III amino-terminal propeptide (P-III-NP) were measured throughout the treatment and washout periods using commercial immunoassays. These analytes were chosen as initial candidate markers because they have previously been used to detect exogenous GH administration (1). Maximal intra-individual changes in IGF-I and P-III-NP were calculated and treatment groups were compared with placebo group using unpaired *t*-tests.

Results: Serum IGF-I and P-III-NP rose in response to rhIGF-I/rhIGFBP-3 administration in both women and men. In women, the mean maximal recorded increase in IGF-I was 993.5±50.4 mg/L (relative increase 373±36%, *P*<0.001 compared with placebo). In men, the mean maximal increase in IGF-I was 751.8±40.4 mg/L (relative increase 318±19%, *P*<0.001 compared with placebo). In women, the mean maximal increase in P-III-NP was 0.158±0.030 U/ml (relative increase 35±7%, *P*=0.034 compared with placebo). In men, the mean maximal increase in P-III-NP was 0.170±0.019 U/ml (relative increase 37±3%, *P*=0.001 compared with placebo). P-III-NP increased and decreased more slowly than IGF-I, although both biomarkers remained elevated in the week after treatment had been completed.

Conclusions: Serum IGF-I and P-III-NP are useful markers of IGF-I abuse. Further IGF/IGFBP axis peptides and markers of bone and collagen turnover will now be evaluated in combination with IGF-I and P-III-NP to develop a robust test for detecting IGF-I abuse by elite athletes.

(1) Powrie JK et al., Growth Horm IGF Res 2007; 17:220

Sources of Research Support: The World Anti-Doping Agency (WADA).

Nothing to Disclose: NG, IE-M, SPN, MF, CB, DC, EEB, PHS, RIGH

Pub # P1-154

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title DNA Methylation at the 11p15 Locus in 20 Monozygotic Twin Pairs after Severe Feto-Fetal Transfusion Syndrome

Author String F Schreiner, O El-Maarri, B Gohlke, S Stutte, P Bartmann, J Oldenburg, J Woelfle
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Body

Background: IGF2/H19 imprinting defects at the 11p15 chromosome region can result in either congenital overgrowth (Beckwith-Wiedemann syndrome) or a growth restriction phenotype (Silver-Russell syndrome). Recent studies also indicate a plasticity of the 11p15 epigenotype in response to environmental changes during early stages in human development.

Study design: We analyzed methylation levels at different 11p15 loci in a cohort of 20 monozygotic twin pairs (mean age 4 years) who were discordant for intrauterine development due to severe feto-fetal-transfusion syndrome (FFTS). This condition had been treated by laser coagulation of anastomosing placental vessels before 25 weeks of gestation. Methylation levels at several CG dinucleotides within the H19 and IGF2 differentially methylated regions (DMR) and the KCNQ1OT1 promoter were determined in DNA samples from blood and buccal cells by the quantitative SNUPE-IP-RP HPLC assay.

Results: Across the entire cohort, we observed a substantial variation in methylation levels at all three regions analyzed. The lowest variation was detected at the KCNQ1OT1 promoter in blood derived DNA (methylation range 42.3 - 47.4 %, median 45.3 %), the highest variation was seen at the IGF2 DMR in buccal cell DNA (range 19.5 - 51.9 %, median 38.3 %). Overall, variation appeared to be markedly higher in buccal cell than in blood cell derived DNA samples. Methylation levels at both H19 and IGF2 DMR showed distinct intra-twin-pair-correlations, confirming previous reports on a strong heritability of the methylation pattern at these two regions (Heijmans et al., Hum Mol Genet 2007). However, with the exception of a weak positive correlation between birth weight-SDS and IGF2 DMR methylation across the entire cohort (40 children; $R=0.31$, $p=0.055$) and an increasing intra-twin-pair variation for IGF2 DMR methylation levels with increasing birth-weight-SDS difference ($R=0.51$; $p=0.023$), we were not able to detect clear methylation differences with regard to the FFTS status (donor vs. recipient). There were also no significant associations between methylation levels and postnatal growth parameters.

Conclusion: In a cohort of 20 growth-discordant monozygotic twin pairs, severe alteration in placental blood supply at least during very early stages in human development (< 25 weeks of gestation) does not seem to leave a persistent epigenetic mark at the analyzed differentially methylated regions at 11p15.

Nothing to Disclose: FS, OE-M, BG, SS, PB, JO, JW

Pub # P1-155

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Impact of IGFBP-3 in Human Metabolic Syndrome: IGFBP-3 Inhibits Cytokine-Induced Insulin Resistance and Early Manifestations of Atherosclerosis in an *In Vitro* System

Author String L Mohanraj, W Li, Y Oh
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Body
Metabolic syndrome is primarily associated with visceral obesity, increased incidence of insulin resistance and cardiovascular diseases. Adipocytes, forming the visceral fat tissue, secrete various adipokines that create a state of local inflammation. This further leads to a state of systemic inflammation, causing insulin resistance and accelerating the events leading to cardiovascular diseases (CVD).
One of the most common manifestations of the metabolic syndrome is insulin resistance. Adipocytes that are exposed to cytokines such as TNF- α activate the NF- κ B signaling cascade. TNF- α treatment in adipocytes resulted in impairment of the IR signaling due to suppression of IRS-1 and GLUT-4 production and alteration of adipokines i.e. decreasing adiponectin and increasing MCP-1 levels. TNF- α is also known to be involved in the atherosclerotic process. NF- κ B regulates adhesion molecules like ICAM and VCAM and chemokines such as MCP-1 that contribute towards CVD. Another well characterized marker of cardiovascular risk is CRP that also increases NF- κ B activity.
IGFBP-3, a major binding protein of IGF modulates IGF by regulating IGF binding to its receptors. It also contributes to the pathophysiology of a variety of disease states in an IGF independent manner. With respect to IGF-I/IGF-I receptor independent actions of IGFBP-3, it is known to interfere and inhibit the NF- κ B pathway. Since NF- κ B plays a significant role in the obesity and progression of atherosclerosis, we explored the role of IGFBP-3 as a therapeutic target for metabolic syndrome.
In human primary adipocytes, IGFBP-3 leads to complete inhibition of TNF- α action and restores the levels of IRS-1, GLUT-4 and adiponectin and lowers MCP-1 levels. IGFBP-3 also sensitizes adipocytes to insulin action seen by increased glucose uptake. Furthermore, in primary human aortic endothelial cells (HAEC), we demonstrated that IGFBP-3 inhibits the elevated NF- κ B activity induced by treatment with factors involved in CVD such as TNF- α , CRP and high glucose. This subsequently led to the inhibition of NF- κ B regulated proteins such as ICAM, VCAM and MCP-1 at mRNA and protein levels thereby inhibiting adhesion of monocytes to HAECs. Taken together, our findings strongly suggest that IGFBP-3 increases insulin sensitivity and may prevent/inhibit the initiation/progression of CVD and pose as an ideal therapeutic candidate for obesity induced CVD and metabolic syndrome in general.

Nothing to Disclose: LM, WL, YO

Pub # P1-156

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Development of a Highly Sensitive and Precise Insulin-Like Growth Factor Binding Protein-3 (IGFBP-3) Immunoassay on the IDS-iSYS System

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Body

INTRODUCTION: Insulin-like growth factor binding protein-3 (IGFBP-3) is a glycoprotein of 45 kDa produced by the liver. It is the most abundant of a group of IGFBPs that transport and control bioavailability and turnover of insulin-like growth factors (IGFs). In the circulation of normal healthy adults, most IGFs are bound in a 150 kDa ternary complex with IGFBP-3 and a 85 kDa acid-labile subunit. The synthesis of IGFBP-3 is primarily controlled by Growth Hormone (GH). Unlike GH secretion, which is pulsatile and demonstrates significant diurnal variation, IGFBP-3 and IGF-1 levels show only minor fluctuations. IGFBP-3 serum level therefore represents a stable measurement of GH production and tissue effect. Low IGFBP-3 and IGF-1 levels are observed in GH-deficiency. Elevated serum IGFBP-3 and IGF-1 levels indicate a sustained overproduction of GH or excessive rhGH therapy. IGFBP-3 has been suggested as a potential prognostic tumor marker.

Objective: We describe the development of a chemiluminescence immunoassay to measure serum levels of the IGFBP-3 on the IDS-iSYS system. This study characterizes the assay's analytical performances.

Methods: IDS-iSYS IGFBP-3 assay uses two highly specific monoclonal antibodies: One coupled to biotin and the second coupled to an acridinium ester derivative. The sample is diluted by the analyzer. 6 [micro]L of the diluted fraction is incubated with both antibodies. After an incubation of 20 min, 20 [micro]L of streptavidin-coupled magnetic particles are added. After a second 10 min incubation, the mixture is submitted to a washing step. Luminescence triggers are then added and the signal obtained is proportional to the amount of IGFBP-3 present in the sample. Sensitivity, imprecision and linearity were determined according to CLSI guidelines.

Results: The IDS-iSYS IGFBP-3 has a dynamic range of 100 to 10000 ng/mL. The LOD and LOQ are respectively below 30 and 80 ng/mL. The assay shows no hook effect up to 130000 ng/mL. The within run CV and total CV of the assay are respectively 3% and 6.4%. There is no detectable cross reactivity with Insulin, IGF-I, IGF-II and the five other IGFBPs. The recovery and linearity are respectively 95% and 106%. 136 samples were tested in comparison with Immulite. Passing-Bablok regression shows IDS-iSYS= 0,97 Immulite + 12 and R=0,97.

Conclusion: The data demonstrate that the IDS-iSYS IGFBP-3 assay is a sensitive and precise method for measuring IGFBP-3 in serum or plasma with excellent correlation with Immulite.

Disclosures: MB: Employee, Immunodiagnostic Systems Ltd. JM: Employee, Immunodiagnostic System Ltd. AKB: Employee, Immunodiagnostic System Ltd. CH: Employee, Immunodiagnostic Systems. MLG: Employee, Immunodiagnostic Systems. MB: Consultant, Immunodiagnostic Systems Ltd.

Pub # P1-157

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title Induction of Apoptosis by Laminarin from *Laminaria digitata* Regulates the IGF-IR Signaling Pathway in Human Colon Cancer Cells

Author String H-K Park, I-H Kim, J Ryu, Y-H Choi, T-J Nam
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Body Growth factor including insulin-like growth factor-I (IGF-I), can potently activate PI3K/Akt signaling, promotes growth factor mediated cell proliferation, differentiation, transformation, and blocks apoptosis. The aberrant activation of the IGF-IR induces growth, neoplastic transformation, and tumorigenesis. The critical role played by the IGF-IR in the development of tumors suggests that this receptor might be an attractive target for dietary intervention for cancer prevention. The laminarin is found in marine brown algae with potential biological activities. Algae in recent years are highlighted as an anticancer medicine and laminarin is a tropical plant traditionally used Chinese medicine. But laminarin has not been investigated for biological activities. In this study, we examined how laminarin from *Laminaria digitata* regulates HT-29 cell and the influence of laminarin from *Laminaria digitata* on the IGF-IR signaling pathway. Using the MTS assay, we obtained laminarin from *Laminaria digitata* induced cell death in a dose-dependent manner. The Western blotting revealed that decreased the phosphorylation of MAPK and ERK. Decreased proliferation was dependent on IGF-IR, which was localized to downregulated MAPK/ERK. These results have important implications for understanding the roles of IGF-IR in colon cancer cell tumorigenesis. Therefore, laminarin could be a potential source of bio-functional food to have anticancer effect in human colon cancer.

Nothing to Disclose: H-KP, I-HK, JR, Y-HC, T-JN

Pub # P1-158

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - IGF & IGF Binding Proteins (1:30 PM-3:30 PM)

Title The Effects of Hot Water Extract from *Chlorella vulgaris* on the IEC-6 Cells' Proliferation

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Body *Chlorella vulgaris*, a unicellular microalgae, exerts various biological effects, but proliferation signaling pathways on normal cells had not been studied. We investigated the effect of hot water extract of *Chlorella vulgaris* (CVE) on cell proliferation and the related signaling pathways in rat intestinal epithelial cells (IEC-6). CVE increased the expression of insulin like growth factor-I receptor (IGF-IR) and the phosphorylation of focal adhesion kinase (FAK) and Src. In addition, CVE induced activation of mitogen-activated protein kinase (MAPK) pathway and phosphatidylinositol 3-kinase (PI3K)/Akt pathway. As a result of experiment, we verified the increased phosphorylation of extracellular-signal related kinase (ERK), Akt and the increased expression of p85. CVE also influence on canonical Wnt pathway, so we found the increase expression of nuclear β -catenin, cyclin D1. Tyr-397 site of FAK can mediate interactions with Src homology 2 (SH2) domains of a number of other signaling proteins including PI3K, PLC- γ , Shc, Grb7, Src and Nck2. Because CVE induced FAK activation, we supposed that FAK could effect on canonical Wnt pathway. As a result of experiment, FAK inhibitor decreased the expression of nuclear β -catenin, cyclin D1, c-myc, and it increased the expression of cytosol β -catenin. We conclude that CVE stimulate IEC-6 cells' proliferation via MAPK pathway, PI3K/Akt pathway and canonical Wnt pathway, and FAK activation influence on canonical Wnt pathway.

Nothing to Disclose: S-HS, I-HK, JR, Y-HC, T-JN

Pub # P1-159

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Inhibins, Activins & TGFbeta Superfamily (1:30 PM-3:30 PM)

Title Myostatin Regulation of Primary Trout Myosatellite Cells: Evidence for Subfunctionalization in a Novel Model System

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Body Myostatin is a potent negative regulator of skeletal muscle growth in mammals. Despite high conservation among vertebrate genes, myostatin function in non-mammalian species is poorly defined. Rainbow trout are unique sources of primary myosatellite cells (a.k.a. skeletal muscle stem cells) as unlike mammals, fish myosatellite cell populations are constantly maintained and significantly contribute to hyperplastic muscle growth even in adults. Compared to mammalian myosatellites, trout cells are easily isolated and can be obtained at far greater numbers. These cells were therefore used to determine the effects of myostatin on proliferation and differentiation. Unlike immortalized mammalian myoblast cell lines, primary trout myosatellites readily differentiate in the presence of serum rather than with serum withdrawal. In fact, differentiation rate was assessed by Myf5, MyoD1, myogenin and MLC expression and was positively correlated to serum concentration. Expression of the different myostatin paralogs increased during differentiation with rtMSTN-1a levels higher than those for -1b and -2a. The rtMSTN-2a transcript, however, was not processed, indicating that autocrine control of myogenesis is regulated by rtMSTN-1 genes and not rtMSTN-2a. Both IGF-I and myostatin temporally stimulated differentiation marker expression, although myostatin's effects were much more pronounced. This differs from studies with mammalian myoblast cell lines where myostatin suppresses rather than stimulates differentiation. Myostatin also suppressed basal and IGF-stimulated proliferation and upregulated rtMSTN-1a expression, which was several fold higher than rtMSTN-1b and -2a. The rtMSTN-2a transcript was always unspliced except in proliferating cells stimulated with IGF-I. Thus, IGF-stimulated differentiation appears to be regulated by the alternative processing of rtMSTN-2a whereas normal differentiation appears to be primarily regulated by the autocrine expression of rtMSTN-1a. These studies are the first to demonstrate myostatin regulation of fish myogenesis. They also suggest that alternative processing of the different myostatin transcripts contributes to gene family subfunctionalization. Furthermore, the general understanding of myostatin action in immortalized mammalian cell lines may not reflect its actions in primary cells as studies with fish and mammalian myosatellites suggest that myostatin activates, rather than inhibits, differentiation.

Nothing to Disclose: DKG, BDR

Pub # P1-160

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Inhibins, Activins & TGFbeta Superfamily (1:30 PM-3:30 PM)

Title The Myostatin Null Phenotype in Senescent Mice; Guarded Enthusiasm

Author String MF Jackson, D Garikipati, OL Nelson, D Luong, JB Stanton, BD Rodgers
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Body The natural aging process results in the physiological decline of multiple tissues and organ systems. Common changes include decreased skeletal muscle mass, bone mineral density, cardiac output, insulin sensitivity and increased adiposity, all of which can contribute to the onset of sarcopenia, osteoporosis, heart failure or type 2 diabetes. Recent studies suggest that the myokine, myostatin, may influence many of these systems directly or indirectly in young mice. We therefore sought to determine whether they are also affected in aging *mstn*^{-/-} mice (12 - 20 m.o.). Body weights were similar in wild-type and *mstn*^{-/-} mice, however, lean fat-free mass and the weights of type I, II and mixed skeletal muscle fibers were significantly greater in the latter. These differences were accompanied by lower total adiposity, weights of individual fat pads (subscapular, inguinal, gonadal & brown) and white and brown adipose cell size in *mstn*^{-/-} mice, although brown fat was only different in female mice. Aged *mstn*^{-/-} mice also responded better to cardiac isoproterenol stress tests with greater increases in fractional shortening and ejection fraction and exhibited several aspects of physiological cardiac hypertrophy that were particularly apparent in female mice. Reduced bone mineral content and a generalized splenic and kidney atrophy also occurred in *mstn*^{-/-} mice, suggesting that the increases in striated muscle mass and function occur at a cost to other systems. Nevertheless, these data suggest that attenuating myostatin could potentially improve the clinical treatment of many pathological conditions that afflict the elderly.

Nothing to Disclose: MFJ, DG, OLN, DL, JBS, BDR

Pub # P1-161

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Inhibins, Activins & TGFbeta Superfamily (1:30 PM-3:30 PM)

Title Activin A Induction of Murine Follicle-Stimulating Hormone β Transcription Is TAK1 (MAP3K7)- and p38 MAPK-Independent

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Body Activins stimulate pituitary follicle-stimulating hormone (FSH) by promoting FSH β subunit (Fshb) gene transcription. Like other transforming growth factor β superfamily (TGF β) ligands, activins signal via heteromeric type I and II receptor complexes. Classically, activated type I receptors phosphorylate intracellular SMAD proteins, which accumulate in the nucleus to regulate target gene transcription. Using immortalized murine gonadotropes (L β T2), we and others demonstrated necessary roles for SMADs 2, 3, and 4 in activin A-stimulated murine and porcine Fshb transcription. In contrast, activins were suggested to regulate the ovine Fshb promoter via a SMAD-independent pathway involving TGF β associated kinase 1 (TAK1, MAP3K7) and p38 MAPK. Here, we examined roles for TAK1 and p38 in activin A-stimulated murine Fshb transcription. In contrast to an earlier report, the TAK1 inhibitor 5Z-7-Oxozeanol (Oxo) did not significantly inhibit the fold activin A induction of murine or ovine Fshb promoter reporters (Fshb-luc) in L β T2 cells, at concentrations up to 5 [μ M] (IC₅₀ = 50-100 nM). Oxo also failed to inhibit activin A-stimulated Fshb mRNA levels or constitutively active activin type I receptor (ALK4)-stimulated Fshb-luc activity. However, 500 nM Oxo attenuated TAK1/TAB2 stimulation of the p38-dependent reporter, GAL4-CHOP, in the same cells. A Map3k7 siRNA similarly antagonized TAK1/TAB2-stimulated GAL4-CHOP activity, but failed to antagonize activin A-stimulated Fshb-luc. Though TAK1 was previously suggested to act via p38 to stimulate the ovine Fshb promoter, we failed to observe activin A-stimulated p38 phosphorylation in L β T2 cells. In apparent contrast, however, the p38 inhibitors SB203580 and SB202190 concentration-dependently attenuated activin A-induced Fshb-luc activity, consistent with the earlier report. Given the lack of p38 activation, we postulated that the inhibitors might non-selectively antagonize ALK4. Indeed, SB202190 attenuated activin A-stimulated SMAD2 phosphorylation, suggesting direct antagonism of ALK4 kinase activity. Collectively, these data suggest that activin A does not signal via TAK1 or p38 to regulate murine Fshb transcription in L β T2 cells and that previous evidence for their involvement might have reflected non-specific effects of the small molecule kinase inhibitors.

Sources of Research Support: Canadian Institutes of Health Research operating grant MOP-89991 to DJB. DJB is a Chercheur boursier senior of the Fonds de la recherche en santé du Québec.

Nothing to Disclose: YW, DJB

Pub # P1-162

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Inhibins, Activins & TGFbeta Superfamily (1:30 PM-3:30 PM)

Title Normal Gonadotropin Production and Fertility in Gonadotrope-Specific Bmpr1a Knockout Mice

Author String X Zhou, Y Wang, U Boehm, Y Mishina, DJ Bernard
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Body Pituitary follicle-stimulating hormone synthesis and secretion are regulated by transforming growth factor β superfamily ligands; most notably the activins and inhibins. Recently, we and others demonstrated that bone morphogenetic proteins regulate FSH β subunit (Fshb) expression in immortalized murine gonadotrope cells (L β T2) and in murine or ovine primary pituitary cultures. Depending on the species and specific ligand, BMP either stimulate or suppress FSH synthesis and secretion. In L β T2 cells, we showed that BMP2 stimulates Fshb transcription in synergy with activin A. Our data further suggested that BMP2 signals preferentially via the BMP type I receptor, BMPR1A. To assess an in vivo role for this receptor in gonadotrope development and/or adult function, we generated gonadotrope-specific Bmpr1a knockout mice (hereafter cKO) using the Cre/loxP system. Bmpr1a^{+/-} are viable and fertile. These animals were mated to GnRH receptor IRES Cre (GRIC) mice, which express Cre recombinase selectively in pituitary gonadotropes and male germ cells. Resulting Bmpr1a^{+/-}; GnhrGRIC^{+/+} females were mated to Bmpr1aloxP/loxP males to generate animals with one of four genotypes. Of these, two were analyzed further: Bmpr1a^{+/-}/loxP; GnhrGRIC^{+/+} (hereafter controls) and Bmpr1a^{-/-}/loxP; GnhrGRIC^{+/+} (cKO). Animals appeared to develop normally and were first analyzed at 6 weeks of age. Body and reproductive organ weights (testes, ovaries, and uteri) did not differ between control and cKO animals. PCR analysis of several tissues confirmed that recombination was restricted to pituitary and testes/epididymides. Serum FSH and LH levels were higher in males than females, but did not differ between genotypes. Similarly, pituitary Fshb and Lhb mRNA levels showed the predicted sex differences, but did not differ between control and cKO mice. Next, 7-10 week old animals of both genotypes and sexes were entered into 6 month mating trials with wild-type C57BL6/J animals. All mice were fertile and no differences were observed between genotypes with respect to days to first litter, number of litters, pups per litter, or inter-litter interval. Collectively, these data suggest that BMPR1A is dispensable for normal gonadotrope development and adult function in mice. As BMPs can signal via multiple type I receptors, Bmpr1a ablation may be compensated for by BMPR1B or ACVR1. Future studies will address this possibility.

Sources of Research Support: Operating grants from the Canadian Institutes of Health Research to DJB (MOP-86626), the Deutsche Forschungsgemeinschaft (DFG) to UB (BO1743/2), and NIH/NIDCR to YM (R01DE02843). DJB is a Chercheur boursier senior of the Fonds de la recherche en santé du Québec.

Nothing to Disclose: XZ, YW, UB, YM, DJB

Pub # P1-163

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Inhibins, Activins & TGFbeta Superfamily (1:30 PM-3:30 PM)

Title The Bone Morphogenetic Protein Type 2 Receptor (BMPR2) Binds Activin A and Mediates Its Actions in Immortalized Gonadotrope Cells

Author String C Rejon, MA Hancock, TE Hebert, DJ Bernard
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Body Activins are members of the TGFβ superfamily. Though originally identified as stimulators of pituitary follicle-stimulating hormone (FSH) synthesis and secretion, they play diverse biological roles, ranging from control of cellular differentiation to regulation of immune responses. Activins exert their actions by binding to complexes of two type I (ACVR1B or ACVR1C) and two type II (ACVR2 or ACVR2B) serine-threonine kinase receptors. Activins bind to the type II receptors, which trans-phosphorylate and activate the type I receptors. The type I receptors then phosphorylate the intracellular signaling proteins, SMAD2 and SMAD3, which form hetero-oligomeric complexes with the co-factor, SMAD4, accumulate in the nucleus, and regulate target gene transcription. TGFβ superfamily ligands greatly outnumber their receptors. Therefore, receptors are shared between ligands and individual ligands can bind multiple receptors. For example, bone morphogenetic proteins (BMPs) can bind and signal via either the bone morphogenetic protein type II receptor (BMPR2) or ACVR2. Here, we asked whether activin A could similarly bind and signal via BMPR2. First, four type II receptors [ACVR2, AMHR2, BMPR2 (long and short isoforms), and TGFBR2] were individually over-expressed in Chinese hamster ovary cells. [¹²⁵I]-activin A bound to ACVR2 and both BMPR2 isoforms but not AMHR2 or TGFBR2, as revealed by affinity labeling. Low nanomolar binding of Activin A to the BMPR2 extracellular domain (ECD) was confirmed by surface plasmon resonance (SPR); however, the apparent affinity was approximately 10-fold weaker compared to the ACVR2 ECD. Nonetheless, the BMPR2 ECD concentration-dependently inhibited activin A- but not TGFβ-induced promoter-reporter activity in immortalized murine gonadotropes (LβT2) cells. Finally, we assessed the functional significance of activin A binding to BMPR2 using RNA interference. Activin A-stimulated FSHβ subunit transcription was attenuated by short-interfering RNAs directed against either *Acvr2* or *Bmpr2*. Conversely, BMPR2 over-expression potentiated the activin A response, and this depended on the kinase activity of the receptor. Taken together the data suggest that BMPR2 represents a functional type II receptor for activin A in gonadotropes. Currently, we are examining potential BMPR2/ACVR2 heterodimerization as well as identifying key residues in BMPR2 that mediate binding to activin A.

Sources of Research Support: Operating grants from the Canadian Institutes of Health Research to DJB (MOP-89991 and MOP-86626) and TEH (MOP-36370). DJB is a Chercheur boursier senior of the Fonds de la recherche en santé du Québec. TEH is a Chercheur national of the Fonds de la recherche en santé du Québec. CR received salary support from the McGill Drug Discovery Training Program.

Nothing to Disclose: CR, MAH, TEH, DJB

Pub # P1-164

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Inhibins, Activins & TGFbeta Superfamily (1:30 PM-3:30 PM)

Title Follistatin: Pattern of Secretion, Correlations with Metabolic Parameters and Its Response to Energy Deprivation and Leptin Administration in Males

Author String MT Vamvini, KN Aronis, JP Chamberland, CS Mantzoros
Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Harvard School of Public Health, Boston, MA; Boston VA Healthcare System, Boston, MA

Body
Aim: Follistatin (FST), a myostatin antagonist, affects muscle mass and is expected to promote glucose uptake and improve glycemic control. It remains unknown whether FST levels display circadian pattern, whether they are associated with glycemic control and whether they are altered by energy deprivation. We first examined the presence of circadian pattern of FST in serum. We then performed the first cross sectional study in humans to determine whether FST is associated with glucose and other metabolic parameters. We then examined the effect of energy deprivation on FST levels and whether this response is mediated by changes in leptin.
Methods: In our cross sectional study a total of 189 men were examined and metabolic/ anthropometric measurements were performed. In the interventional study we studied in our GCRC 7 healthy lean men for 72 hours each time under 3 different conditions: baseline study in the isocaloric (fed) state and 2 complete fasting studies with administration of either placebo or replacement dose of recombinant methionyl human leptin. We measured levels of leptin (RIA) and FST (ELISA) in samples obtained hourly for 24hrs from day 3 to day 4. Statistical analysis: Cross-Sectional Study: Pairwise associations were estimated with a Pearson's correlation. Adjustment for age, BMI, urea, GFR was performed running multivariate analysis. The existence of underline periodicity was examined with nonlinear four-parameter cosine OLS regression. The FST levels between the conditions were compared using a Hierarchical Mixed-Effects Linear Modeling approach.
Results: The cross sectional study showed a strong correlation between FST and glucose levels ($p=0.0003$, $r=-0.266$). No significant correlation was found between FST and lipid profile of the subjects before and after adjustment for potential confounders. The circadian rhythm analysis showed the absence of a consistent day night pattern of FST secretion. There was significant increase by $\sim 165\%$ in FST levels in acute energy deprivation ($p < 0.001$) which was not corrected in response to administration of replacement dose of leptin.
Conclusions: Our data did not demonstrate a day -night pattern of FST secretion. The effect of energy deprivation to increase FST levels is not mediated through leptin. FST correlates strongly with glucose levels consistent with findings from animal studies suggesting that FST acts as a myostatin antagonist to increase muscle mass, glucose uptake and improve glycemic control.

Sources of Research Support: National Institutes of Health - National Center for Research Resources grant M01-RR-01032 (Harvard Clinical and Translational Science Center) and grant number UL1 RR025758. The Mantzoros Lab is also supported by the National Institute of Diabetes and Digestive and Kidney Diseases grants 58785, 79929 and 81913, and AG032030. Amylin Pharmaceuticals, Inc. supplied metreleptin for this study but had no role in the study design; conduct of the study; collection, management, analysis, and interpretation of the data; or the preparation, review, or approval of the manuscript.

Nothing to Disclose: MTV, KNA, JPC, CSM

Pub # P1-165
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)
Title Defining Dimerization Sites on the TSH Receptor Transmembrane Domain
Author String R Ali, R Latif, M Mezei, TF Davies
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Body The thyroid stimulating hormone receptor (TSHR), an important member of the GPCR protein family, has not been fully modeled. Only the crystal structure of the ecto-domain has been reported. Biophysical (FRET) and biochemical studies from our laboratory have established that the TSHR, similar to other GPCRs, has the propensity to form dimers and multimers both in native and non-native cells. While our recent experimental studies (1) have shown that dimerization interfaces can reside in the extracellular domain, our experimental data with truncated TSHRs have indicated that the trans-membrane (TM) region has the major role in dimerization and multimerization of this receptor. For example, the TSHR devoid of its ectodomain shows similar multimerization characteristics as the full length receptor. We have used Brownian dynamics in predicting transmembrane dimerization, a well established technique for examining such biological phenomena. The method was implemented in Macrodox and involved complementing the Poisson-Boltzmann equation based calculation of the electrostatic interactions with the calculation of Van der Waals interactions (2). As dimerization occurs in the membrane bilayer, we have restricted our trans-membrane monomers' movements to the membrane plane. We retained monomer I centered at the origin and subjected monomer II to three forces (electrostatic, van der Waals, and the frictional force due to solvent viscosity). Several independent runs were performed and from each run the dimers that had the lowest energy and the closest approach were saved. Of all dimers saved, the two lowest energy conformations showed that TM-1 (29-Leu) makes contact with TM-6 (152-Val) in one conformation while another structure shows contact between TM-1 (26-Leu) and TM-7 (196-Ile). Experimental data, deleting TM-1 from a full length TSHR construct, confirmed that TM-1 is essential for full TSHR multimerization and added validity to our in-silico observations. These data show that Brownian dynamics can predict TSHR interfaces and receptor-receptor interactions.

(1) Latif R, Michalek K, Morshed SA, Davies TF. PLoS One 2010
26:e9449

(2) Cui M, Mezei M, Osman R. Journal of Computer-Aided Molecular Design 2008 22:553

Nothing to Disclose: RA, RL, MM, TFD

Pub # P1-166
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)
Title Conformational Epitope Mapping of Thyroid-Stimulating Antibody MS-1 Using Residue Protection and Dual Mass Spectrometry
Author String R Latif, A Teixeira, M Schlesinger, SA Morshed, K Michalek, TF Davies
Mount Sinai School of Medicine, New York, NY; James J Peters VA Medical Center, New York, NY;
University of Medical Sciences, Poznan, Poland

Body The thyrotropin receptor (TSHR), the major autoantigen in Graves' disease, triggers the immune system to produce pathogenic TSHR antibodies which may act as thyroid stimulators of varying potency. The binding and activity of stimulating antibodies are conformation dependent and the detailed mapping of their epitopes is needed in order to understand their structure/function relationships. The aim of this study was to identify the conformational epitopes of our well characterized hamster monoclonal thyroid stimulating antibody MS-1 on the entire ectodomain of the human TSH receptor by using the method of epitope protection. Conformational epitopes consist of several short linear peptides that are spatially organized to form the antigenic determinants. By first protecting the receptor with antibody prior to digestion the protected peptides can be delineated by mass spectrometry. Chinese hamster ovary (CHO) cells stably expressing the entire ectodomain (TSHR-ECD, aa1-412) attached to a GPI linker was used as the source of the TSHR antigen. Purified TSHR-ECD was obtained by digesting with enzyme (PI-PLC) which specifically cleaved the GPI linker and released soluble TSHRs. This soluble TSHR-ECD retained its native structure as evidenced by binding of MS-1 in immunoprecipitation assays. The MS-1/TSHR-ECD complex was then captured by a protein G Sepharose column and the antibody was covalently cross-linked. The bound complex of MS-1/TSHR-ECD was then digested with trypsin in a time dependent manner. The unbound digested fragments were washed out and the remaining receptor fragments were eluted and analyzed in a mass spectrometer (LC/MS/MS). The peptide sequences obtained by the LTQ ion trap nanospray were compared to the theoretical digest of the TSHR ECD. An isotopic antibody and antibodies with known linear and conformational epitopes were used as controls. This epitope protection approach revealed the presence of 7 major individual linear peptides spanning residue 18 to 291 of the TSHR-ECD. Five of these regions corresponded to peptides in leucine rich domains (LRD) 2, 5, 8, 9 and 10. In addition, TSHR-Ab MS-1 contacted a 19aa region on the amino half of the TSHR hinge region (274-293) which encompasses a highly conserved motif (SHCCAF) previously implicated in activation of the receptor. These data demonstrate that stimulating TSHR-Abs have epitopes not confined to the LRD but incorporate the hinge region of the TSH receptor.

Sources of Research Support: NIH Grant DK069713 and VA Merit Award program 4535-05-07 awarded to TFD.

Nothing to Disclose: RL, AT, MS, SAM, KM, TFD

Pub # P1-167

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)

Title Recycling of Internalized TRH Receptors Requires Receptor Dephosphorylation

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University of Rochester Medical School, Rochester, NY

Body Following activation, many G protein-coupled receptors (GPCRs) become phosphorylated, bind arrestin and undergo endocytosis. Agonist-dependent receptor internalization plays a key role in desensitization. Internalized GPCRs eventually become degraded or recycle to the plasma membrane, where they can signal again. The factors determining the fate of internalized GPCRs are not well understood. In this study, we have asked whether dephosphorylation of internalized, phosphorylated TRH receptors is required for recycling and resensitization. After binding TRH, the Gq/11-coupled TRH receptor is rapidly phosphorylated by GPCR kinase 2 (GRK2). The activated and phosphorylated receptor recruits arrestin to the plasma membrane and internalizes with it via clathrin-coated pits. We used affinity-purified antibodies that bind specifically to phosphorylated TRH receptors to quantify GRK phosphorylation and identify phosphoreceptors by fluorescence microscopy. To follow receptor trafficking, we studied cells stably expressing TRH receptors tagged at the extracellular N-terminus with an HA-epitope and applied mouse anti-HA antibody to intact cells such that only receptors on the surface at the beginning of the experiment were labeled. TRH receptors became phosphorylated in seconds and internalized in minutes. The receptors remained intracellular as long as extracellular TRH was present. Following a 30 min exposure to TRH, receptors were in deep endosomes where they remained phosphorylated and associated with arrestin. Addition of the inverse agonist chlordiazepoxide triggered dephosphorylation (half-time 10 min) and gradual recycling of HA-tagged receptors to the plasma membrane. To determine whether recycling was initiated by the loss of TRH receptor signaling or the loss of TRH receptor phosphorylation, we screened phosphatase inhibitors and identified conditions where dephosphorylation of the TRH receptor could be inhibited. Treatment with the phosphatase inhibitor calyculin dramatically reduced the rate of dephosphorylation of internalized TRH receptors. At the same time, calyculin blocked the reappearance of internalized receptors at the plasma membrane. These results demonstrate that receptor dephosphorylation is required for TRH receptor recycling, and suggest that the phosphorylation state of the receptor is the critical signal that directs trafficking of intracellular receptors and thereby regulates the resensitization process.

Sources of Research Support: NIH DK19974.

Nothing to Disclose: PMH, JGT, AUG

Pub # P1-168
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)
Title The GPR30 Pathway Is Involved in Mediating the Estrogenic Effects of Ginsenoside Rg1
Author String Q Gao, MS Wong
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Body Ginsenoside Rg1 (an abundant active ingredient in Panax Ginseng) can induce estrogen response element (ERE)-dependent transcription via MAPK kinase (MEK) in ligand-independent activation of estrogen receptor (ER) in estrogen responsive cells. We hypothesize that the G-protein coupled receptor 30 (GPR30) pathway is involved in mediating the estrogenic actions of Rg1. The present study aims to investigate the role of GPR30 involved in mediating the activation of ER by Rg1. Human breast cancer MCF-7 cells (ER positive) were treated with different concentrations of Rg1 (10^{-14} M- 10^{-6} M), 17β -estradiol (E_2 , 10^{-8} M) or its vehicle. To determine if GPR30 was involved in the stimulatory actions of Rg1, MCF-7 cells were pretreated with GPR30 antagonist (G15) for 20min and then co-treated with Rg1. The effects of Rg1 on the cell proliferation were studied by using MTS assay. The protein expression of MEK and phosph-MEK were studied by Western blotting. In addition, the ability to induce ER-dependent transcription was assessed in MCF-7 cells transfected with ERE-luciferase construct or AP1-luciferase construct. Our results suggested that ginsenoside Rg1 could significantly increase the cell proliferation in a dose-dependent manner. Pre-treatment with G15 (10^{-6} M) could significantly block the effects of Rg1 on cell proliferation in MCF-7 cells. Both Rg1 and E_2 could significantly induce MEK phosphorylation in a time-dependent manner without alteration of MEK protein expression. Moreover, Rg1 at 10^{-8} M and 10^{-6} M could significantly increase MEK phosphorylation in MCF-7 cells upon treatment for 30 minutes. Co-treatment of MCF-7 cells with G15 completely abolished the stimulatory effects of Rg1 on MEK phosphorylation. Furthermore, Rg1 (10^{-8} M) and E_2 (10^{-8} M) could significantly induce the ERE-dependent and AP1-dependent transcription in MCF-7 cells. The stimulatory effects of Rg1 on AP-1 dependent as well as ERE-dependent transcription in MCF-7 cells could be blocked by co-treatment with G15. Our results clearly demonstrated that ginsenoside Rg1 could increase cell proliferation, MEK phosphorylation as well as ERE-dependent and AP1-dependent transcription through the GPR30 pathway. The results support our hypothesis that GPR30 pathway is involved in mediating the estrogenic effects of ginsenoside Rg1.

Nothing to Disclose: QG, MSW

Pub # P1-169

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)

Title The Kisspeptin/KISS1R Complex Undergoes Rapid Internalization and Turnover, Which May Contribute to Desensitization and Resensitization

Author String L Min, AC Reis, S Xu, RS Carroll, U Kaiser
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Body Kisspeptin and its receptor, KISS1R, are essential regulators of GnRH neuronal function. Loss-of-function mutations of KISS1R have been identified in patients with GnRH deficiency. Similarly, Kiss1r^{-/-} mice display a phenotype consistent with GnRH deficiency. We have studied the internalization, desensitization, and turnover of the kisspeptin/KISS1R complex, which can have important implications for KISS1R signaling. Using CHO-KISS1R cells, which have stable expression of KISS1R, the fate of internalized ligand was determined by measuring released and internalized 125I-kisspeptin-10. KISS1R was found to internalize rapidly following ligand stimulation, with a half-life (t_{1/2}) of 8 minutes. Pretreatment with an endocytosis inhibitor, phenylarsine oxide, inhibited KISS1R internalization and increased t_{1/2} to 26 minutes, confirming that KISS1R internalization follows a classical endocytosis pathway. Within one hour, more than 70% of the internalized ligand was released, primarily in degraded form, suggesting rapid processing of the internalized kisspeptin/KISS1R complex. This rapid ligand degradation may be a mechanism contributing to kisspeptin desensitization. A proteasome inhibitor (MG132) but not a lysosome inhibitor (chloroquine) reduced the rate of degradation of internalized kisspeptin, suggesting a proteasome-mediated pathway of degradation of the internalized kisspeptin. Since G protein-coupled receptors typically undergo lysosome-mediated degradation, it is not clear from these data if the KISS1R enters the proteasome-mediated degradation pathway together with its ligand or if the KISS1R dissociates from its ligand, kisspeptin, after internalization and then enters either a recycling pathway or a lysosome-mediated degradation pathway. Following trypsin-mediated proteolytic removal of cell surface KISS1R, full replenishment of cell surface KISS1R from the intracellular pool occurred within 10 minutes, suggesting dynamic constitutive KISS1R trafficking between plasma membrane and intracellular KISS1R pools. In summary, our data show both ligand-dependent and -independent robust KISS1R internalization and recycling. Interestingly, the majority of internalized ligand is rapidly degraded which may contribute to desensitization, whereas rapid replenishment of cell surface KISS1R from intracellular pools may contribute to resensitization.

Nothing to Disclose: LM, ACR, SX, RSC, UK

Pub # P1-170
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)
Title Somatostatin Receptor-2 and Opioid Receptor-Mediated Changes in MAPK, Cell Survival Pathways and Tumor Suppressor Proteins in Human Breast Cancer Cells
Author String G Kharmate, PS Rajput, Y-C Lin, U Kumar
University of British Columbia, Vancouver, Canada

Body Somatostatin and opioids are inhibitory regulators of cell growth and their anti-proliferative effects are exerted through G-protein coupled somatostatin receptors (SSTR1-5) and opioid receptors (ORs) including [micro], [delta], and [kappa] respectively. SSTRs and ORs share >40% structural homology and are well expressed in various breast cancer cells as well as in solid tumor tissues at the cellular and molecular levels. Among all SSTRs, SSTR2 subtype is a principal mediator of anti-proliferation on normal as well as tumor cells. Recently, over-expression of SSTR2 in human MCF-7 breast cancer cells induced anti-proliferative effects via both cytostatic as well as cytotoxic manner. In addition to their classic analgesic role, recent studies have suggested that ORs also suppress tumor cell growth upon activation in breast cancer cells. Since both SSTR2 and ORs are variably expressed in breast cancer cells, their analogous structures are indicative of potential functional interactions. However, it is currently unknown whether SSTR2 co-express and functionally interacts with ORs in breast cancer cells. Existing observations suggest that the activation of SSTR2 or ORs results in regulation of downstream signaling pathways including the MAPKs, which leads to the inhibition of cell growth. Accordingly, in the present study, immunocytochemistry and western blot analysis were employed to study the colocalization and changes in MAPKs (ERK1/2 and p38), cell survival pathway (PI3K/AKT) and tumor suppressor proteins (PTEN and p53) in three human MCF7 (estrogen receptor +), MDA-MB231 (ER-), and T47D (ER+) breast cancer cell lines. Our results indicate agonist dependent colocalization and internalization of SSTR2 and ORs in receptor and cell-specific manner. Furthermore, simultaneous activation of SSTR2 and ORs exert anti-proliferative effects via activation of MAPKs and tumor suppressor proteins, in addition to the blockade of cell survival pathway in an ER dependent manner. In conclusion, this is the first comprehensive study describing the SSTR2 and ORs receptor trafficking and modulation of signaling pathways in tumor cells that might have better therapeutic approach in breast cancer treatment.

Sources of Research Support: Canadian Institute of Health Research Grant (MOP 10268 and MOP 74465) and grant from Canadian Breast Cancer Foundation BC/Yukon to UK. UK is a Senior Scholar of Michael Smith Foundation for Health Research.

Nothing to Disclose: GK, PSR, Y-CL, UK

Pub # P1-171
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)
Title Role of Somatostatin in Modulation of β -Adrenergic Receptor-Mediated Downstream Signaling Pathways in H9c2 Cells
Author String RK Somvanshi, X Qiu, U Kumar
University of British Columbia, Vancouver, Canada

Body Somatostatin receptors (SSTRs) and β -adrenergic receptors (β ARs) belong to GPCR family and are well expressed in cells of cardiac origin. SSTRs and β ARs activate different G-proteins and exert distinct physiological effects. β ARs are associated with cardiac hypertrophy in receptor dependent manner whereas, somatostatin (SST) analogues Octreotide and Lanreotide elicits regression of Left ventricular hypertrophy (LVH) as well as in the normalization of cardiac functions. We have recently shown that these receptors functionally interact at the cellular level in transfected cells via heterodimerization and modulate downstream signaling pathways in receptor dependent manner. Whether interactions between SSTRs and β ARs in cardiac cells expressing these receptors endogenously are same or different is largely elusive. In the present study, we investigated the expression of SSTR and β AR subtypes and the role of SST on signaling pathways with or without β_1 and β_2 adrenergic receptor specific agonist in rat fetal cardiomyocytes (H9c2 cells). We determined the effect of SST on signaling cascades associated with cardiac hypertrophy and heart failure including cAMP/PKA, MAPKs (ERK1/2 and p38) and NFAT. Additionally we also determined mitochondrial membrane potential by using JC1 as an index of apoptosis in H9c2 cells. Our findings demonstrate that SSTR subtypes (SSTR1-5) and β ARs (β_1 and β_2 AR) are well expressed in H9c2 cells at the protein level and exhibit receptor specific distributional pattern. SST ameliorate β ARs antagonist effect whereas decreases the effect of β ARs agonist on the intracellular cAMP level in H9c2 cells. Changes in the status of phosphorylated ERK1/2 and p38 indicate that SST antagonizes β_1 AR agonist isoproterenol mediated effect in H9c2 cells. JC1 staining indicates that SST provides defensive mechanism against isoproterenol induced mitochondrial damage and apoptosis. Collectively, our data describe that β_1 AR and β_2 AR stimulated signaling are modulated upon SST treatment in receptor specific manner. SST abrogates β_1 AR mediated changes while enhances β_2 AR mediated signaling pathways. The results presented here are the first direct evidence elucidating the unidentified role of SST in cardiac cells and might provide new therapeutic approach in heart failure.

Sources of Research Support: Canadian Institute of Health Research Grant (MOP 10268 and MOP 74465). UK is a Senior Scholar of Michael Smith Foundation for Health Research.

Nothing to Disclose: RKS, XQ, UK

Pub # P1-172
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)
Title Co-Expression of Human Somatostatin Receptor Subtypes 2 and 3 in HEK-293 Cells Modulates Antiproliferative Signaling and Apoptosis
Author String SA War, U Kumar
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Body G-protein-coupled receptors (GPCRs) are known to assemble as heterodimers with distinct physiological, biochemical and pharmacological properties, and this concept has broadened the therapeutic potential of drug-targeting GPCRs. Somatostatin (SST) is an inhibitory peptide and acts via five G-protein-coupled somatostatin receptors (SSTR1-5) to elicit antiproliferative effects. SSTRs form heterodimers within the same family and also with other GPCRs resulting in activation or inactivation of signaling pathways. SSTR5 and dopamine receptor-2 heterodimerization leads to enhanced functional activity. On the other hand, SSTR2 and SSTR3 heterodimer abrogated SSTR3 functions, however, these studies were performed on receptors of rat origin which exhibit distinct response to agonist when compared to human (h) receptors. Whether hSSTR2 and hSSTR3 interact in a similar manner to modulate antiproliferative signaling is not known. In the present study, we investigated MAPK signaling, cell proliferation and apoptosis in HEK-293 cells stably co-transfected with hSSTR2 and hSSTR3 in response to SST, L-779976 (SSTR2 agonist) and L-796778 (SSTR3 agonist) under normal and stressful (serum-deprived) conditions. p-ERK1/2 inhibition in response to SST and agonists was time-, concentration- and receptor-dependent, and the inhibition was more pronounced in stressful environment. p-JNK was decreased in presence of SST and L-796778 for 30 min, while in contrast, increased with SST and agonists for 24 h. p-p38 levels were significantly increased upon treatment with SST, L-779976 and L-796778 for 30 min, and serum deprivation while elevating basal p-p38 resulted in tapering of agonist-mediated effects. Interestingly, p-p38 was completely lost in cells treated with agonist for 24 h. MTT assay demonstrated antiproliferative effects of SST and specific-agonists, and in agreement, p21 and p27^{Kip1} expression was increased with SST and agonists. TUNEL assay and PARP-1 expression provide evidence for hSSTR2- and hSSTR3-mediated apoptosis, which was enhanced upon co-activating both receptors as well as starvation. Our study suggests that co-expression of hSSTR2 and hSSTR3 modulates MAPKs and enhances antiproliferative signaling. Moreover, the potential role of heterodimerization in mediating these effects cannot be ruled out and further studies are in progress. Taken together, these data provide new insights in understanding the molecular interactions involving SSTRs in tumor biology.

Sources of Research Support: CIHR, CBCF-BC/Yukon, and MSFHR.

Nothing to Disclose: SAW, UK

Pub # P1-173

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)

Title Functional Characterization of Calcium Sensing Receptors (CaSR) Bearing Loss-of-Function or Gain-of-Function Missense Mutations

Author String A Diaz-Thomas, P Iyer, A Root, J Cannon
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Body The CaSR is a G-protein coupled receptor expressed in the parathyroid gland, renal tubule, and other tissues that is responsible for discerning and maintaining normal concentrations of serum ionized calcium (Ca^{2+}). Clinically, inactivating mutations of *CASR* are associated with familial hypocalciuric hypercalcemia/neonatal severe hyperparathyroidism, while activating mutations lead to autosomal dominant hypoparathyroidism. We have explored the functional characteristics of two mutations associated with both disorders. Two Pakistani children whose parents were first cousins and who demonstrated hypercalcemia, hypophosphatemia, and elevated serum levels of parathyroid hormone (PTH) very shortly after birth were homozygous for a novel point mutation in *CASR* (2303G>T) resulting in substitution of valine for glycine at codon 768 (G768V); their parents were asymptomatic heterozygous carriers of this mutation with normal serum levels of calcium, phosphate, and PTH. In order to assess the functional consequence of G768V, the mutated CaSR was expressed as a recombinant, haemagglutinin (HA) epitope-tagged protein in HEK293 cells; its membrane trafficking was tracked by immunofluorescence and its function examined by calcium-stimulated activation of MAP kinase - ERK. Wild type (WT) CaSR and an adjacent CaSR variant (E767Q) associated with autosomal dominant hypoparathyroidism were similarly investigated. Indirect immunofluorescence using fluorescein-tagged antibodies to HA demonstrated that all three variants of CaSR were present on the cell membrane. Neither of the mutated CaSRs was retained within the cell. Preliminary Western blot analyses revealed that both G768V and E767Q CaSR mutants induced ERK phosphorylation in response to stimulation by increasing concentrations of Ca^{2+} , although their responses differed quantitatively from those of WT CaSR. Additionally, there was a quantitative difference between the responses to Ca^{2+} of the two mutants. These data indicate neither the CaSR inactivating mutation G768V nor the activating mutation E767Q interferes with normal membrane trafficking of the mutant protein product. However, the quantitatively different functional responses to varying concentrations of Ca^{2+} likely reflect an intrinsic defect in binding of Ca^{2+} or in the efficiency of signal transduction by the mutant receptors.

Nothing to Disclose: AD-T, PI, AR, JC

Pub # P1-174

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)

Title Apical Membrane Segregation of Phosphatidylinositol(4,5)Bisphosphate Influences Parathyroid Hormone 1 Receptor Compartmental Signaling and Localization Via Direct Regulation of Ezrin in LLC-PK1 Cells

Author String MJ Mahon
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Body The parathyroid hormone 1 receptor (PTH1R), a primary regulator of mineral ion homeostasis, is expressed on both the apical and basolateral membranes of kidney proximal tubules, a localization pattern that is also displayed in the LLC-PK1 kidney cell line. In LLC-PK1 cells, apical PTH1R localization is dependent upon direct interactions with ezrin, an actin-membrane cross-linking scaffold protein. To function as a scaffold, ezrin undergoes an activation process that is dependent upon phosphorylation and binding to phosphatidylinositol(4,5)bisphosphate (PIP2). Recent evidence demonstrates that PIP2 exists in apical membranes of polarized epithelia. Consistently, the intracellular probe for PIP2, GFP-PLCdelta1-PH, localizes to the apical, brush border membranes of LLC-PK1 cells, directly overlapping ezrin and PTH1R expression. Activation of the apical PTH1R shifts the GFP-PLCdelta1-PH probe from the apical membrane to the cytosol and basolateral membranes, likely reflecting the activation of phospholipase (PLC) and hydrolysis of PIP2. PTH1R-mediated accumulation of inositol trisphosphates and activation of MAPK are markedly enhanced when PTH is applied to the apical surface of LLC-PK1 cells as compared to basolateral applications. This compartmental signaling is likely due to the polarized localization of PIP2, the substrate for PLC. PIP2 degradation using a membrane-directed phosphatase shifts ezrin localization to the cytosol and induces ezrin de-phosphorylation, processes consistent with inactivation. PIP2 degradation also shifts PTH1R expression from brush border microvilli to basolateral membranes and markedly blunts PTH-elicited activation of the MAPK pathway with only marginal effects on signaling via Gs/cAMP. Several lines of evidence suggest that the ezrin-based scaffold promotes the formation and stabilization of PIP2 in the apical membranes of polarized epithelia. As a component of this scaffold, the PTH1R is placed in juxtaposition to the substrate of PLC (i.e. PIP2) and thus promotes PTH signaling via this pathway.

Nothing to Disclose: MJM

Pub # P1-175

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)

Title Retromer Terminates the Generation of cAMP by Internalized PTH Receptors

Author String TN Feinstein, VL Wehbi, TJ Gardella, J-P Vilardaga
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Body Generation of cAMP by G protein-coupled receptors (GPCRs) and its termination is currently thought to occur exclusively at the plasma membrane of cells. Under existing models of receptor regulation, this signal is primarily restricted by desensitization of the receptors through their binding to **b**-arrestins. However, this paradigm is not consistent with recent observations that the parathyroid hormone receptor type 1 (PTHr) continues to stimulate cAMP production even after receptor internalization, as **b**-arrestins are known to rapidly bind and internalize activated PTHR. Here we show that **b**-arrestin1 binding prolongs rather than terminates cAMP generation by PTHR, and that cAMP generation correlates with the persistence of arrestin-receptor complexes on endosomes. We found that PTHR signaling is instead turned-off by the retromer complex, which regulates traffic of internalized receptor from endosomes to the Golgi apparatus. Thus, binding by the retromer complex regulates sustained cAMP generation triggered by an internalized GPCR.

Nothing to Disclose: TNF, VLW, TJG, J-PV

Pub # P1-176

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)

Title Dynamic Formation of a PTH/PTHrP Receptor-Arrestin-G $\beta\gamma$ Signaling Complex

Author String VL Wehbi, TN Feinstein, JA Ardura, G Romero, J-P Vilardaga
University of Pittsburgh, School of Medicine, Pittsburgh, PA; Massachusetts General Hospital and Harvard Medical School, Boston, MA

Body Arrestins and heterotrimeric G proteins (G $\alpha\beta\gamma$) regulate G-protein-coupled receptors (GPCRs) signaling and trafficking. Arrestins binding to activated GPCRs terminate receptor and G protein coupling, and promote receptor internalization. The binding of the β -arrestins and G proteins on activated GPCR is thought to be mutually exclusive. Here we show that β -arrestins prolong rather than shut down PTH/PTHrP receptor (PTHR) signaling. By using optical approaches (confocal microscopy, FRAP, TIRF, fluorescence correlation spectroscopy, and FRET) in live cells in real time we found that PTHR forms a ternary complex with β -arrestin1/2 and G $\beta\gamma$ subunits in response to PTH stimulation. We further confirmed the formation of a PTHR-arrestin-G $\beta\gamma$ complex in response to PTH binding by coimmunoprecipitation assays. Additionally, we showed that the rapid ($t_{1/2} < 60$ s) assembly/disassembly dynamics formation of a receptor microdomain that contains PTH, PTHR, arrestin and G $\beta\gamma$ subunits regulates cAMP levels in magnitude and duration. These data raise the novel model whereby the formation of a long-lived PTHR-G $\beta\gamma$ -arrestin ternary complex contributes to prolonged receptor signaling by mechanisms that presumably permit multiple rounds of G α S subunit coupling and activation.

Nothing to Disclose: VLW, TNF, JAA, GR, J-PV

Pub # P1-177

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)

Title β -Arrestin Mediates the Desensitization and Internalization of KISS1R

Author String M Ahow, N Sharma, C Glidewell-Kenney, M Pampillo, D Fraser, PL Mellon, AV Babwah
The Children's Health Research Institute, London, Canada; Lawson Health Research Institute, London, Canada; University of Western Ontario, London, Canada; University of Western Ontario, London, Canada; University of Western Ontario, London, Canada; University of California, San Diego, La Jolla, CA

Body The kisspeptin (Kp)/KISS1R (GPR54) signaling system triggers gonadotropin-releasing hormone (GnRH) secretion from GnRH neurons and thereby acts as a major regulator of the hypothalamic-pituitary-gonadal (HPG) axis. Recent studies have indicated that kisspeptin agonists can serve as highly effective alternatives to GnRH agonists in the treatment of several gynaecological disorders, hormone-dependent cancers, isolated hypogonadotropic hypogonadism and central precocious puberty. Since kisspeptin and GnRH agonists achieve their therapeutic potential by either triggering or delaying the desensitization and internalization of their cognate receptors, we have undertaken studies to identify the mechanisms by which KISS1R undergoes desensitization and internalization. Recently, using cellular models, we demonstrated that KISS1R undergoes rapid homologous desensitization in a G protein-coupled receptor kinase-2 (GRK2)-dependent manner (Pampillo et al., 2009). In the present study, we demonstrate a role for β -arrestin-1 and -2 in KISS1R desensitization and internalization in the continuous presence of its agonist. Using inositol phosphate (IP) formation as a readout of KISS1R activity in three cellular systems, we demonstrate that overexpression of β -arrestin in COS-7 cells reduced IP formation, while the absence and reduced expression of β -arrestin in mouse embryonic fibroblasts (MEFs) and hypothalamic neurons (GT1-7), respectively, resulted in increased IP formation. Using a YFP-tagged KISS1R molecule, we also demonstrate that β -arrestin-1 and -2 are both required for KISS1R internalization since receptor internalization is blocked in MEFs lacking either isoform. To better understand the physiological importance of β -arrestin, we are currently conducting studies in the β -arrestin-downregulated GT1-7 cells to determine what effect reduced β -arrestin expression has on GnRH release. Our findings add significantly to the mechanistic understanding of KISS1R desensitization and internalization and have also identified new targets for the treatment of pathophysiological conditions requiring the modulation of the HPG-axis.

Pampillo et al., Mol Endo 2009; 23: 2060

Sources of Research Support: Grants from the Natural Sciences and Engineering Research Council of Canada 327334-2006 (AB), the Canadian Institutes of Health Research MOP#81383 (AB), U54 HD012303 (PM) R01 DK44838 (PM) and R01 HD020377 (PM).

Nothing to Disclose: MA, NS, CG-K, MP, DF, PLM, AVB

Pub # P1-178
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)
Title Profiling Novel Pharmacology Resulting from G Protein-Coupled Receptor Heteromerization
Author String KD Pflieger, S Mustafa, HB See, RM Seeber, MA Ayoub
Western Australian Institute for Medical Research & Center for Medical Research, University of Western Australia, Nedlands, Perth, Australia

Body The concept that G protein-coupled receptors (GPCRs) have the capacity to form receptor-receptor interactions has been described in the literature for nearly 30 years (see (1) for review). Of most interest is the realization that novel pharmacology can result when receptors form complexes with different receptors (heteromers), a phenomenon that has been termed the 'biochemical fingerprint of the receptor heteromer' (2). We have profiled GPCR heteromers looking at a range of functional endpoints, including coupling to the different G protein and ERK signaling pathways. We have also studied internalization profiles, and utilized the recently published GPCR-Heteromer Identification Technology (GPCR-HIT) (3) to assess pharmacology specifically from the heteromer as opposed to the co-expressed monomers/homomers. We have demonstrated examples of GPCR heteromerization resulting in changes in: G protein-coupling as assessed by measuring inositol phosphate or cAMP production, or Gi-receptor proximity using bioluminescence resonance energy transfer (BRET); β -arrestin recruitment profiles using GPCR-HIT in the BRET configuration; and internalization as assessed by either confocal microscopy or enzyme-linked immunosorbent assay (ELISA). The profiling approach we have adopted enables multiple pathways to be evaluated under similar experimental conditions in order to generate a biochemical fingerprint of the receptor heteromer in a model cell line. These findings can then be linked to phenotypes observed under more physiologically-relevant conditions, including whole animal studies, thereby moving us significantly closer to understanding the mechanism of action of hormones acting on receptor complexes. Crucially, studies can subsequently be extended to evaluate existing and novel pharmaceuticals for a range of endocrine-related disorders, particularly with the aim of better understanding side effect profiles and their underlying mechanisms.

(1) Fuxe, K., Marcellino, D., Borroto-Escuela, D. O., Frankowska, M., Ferraro, L., Guidolin, D., Ciruela, F., and Agnati, L. F. (2010) *J Recept Signal Transduct Res* 30, 272-283

(2) Ferre, S., Baler, R., Bouvier, M., Caron, M. G., Devi, L. A., Durroux, T., Fuxe, K., George, S. R., Javitch, J. A., Lohse, M. J., Mackie, K., Milligan, G., Pflieger, K. D., Pin, J. P., Volkow, N. D., Waldhoer, M., Woods, A. S., and Franco, R. (2009) *Nat Chem Biol* 5, 131-134

(3) See, H. B., Seeber, R. M., Kocan, M., Eidne, K. A., and Pflieger, K. D. (2011) *Assay Drug Dev Technol* ahead of print. doi:10.1089/adt.2010.0336

Sources of Research Support: National Health and Medical Research Council (NHMRC) of Australia (Project Grant 566736); Australian Research Council Future Fellowship (FT100100271) awarded to KDGP; Dimerix Bioscience Pty Ltd.

Disclosures: KDP: Chief Scientific Officer, Dimerix Bioscience Pty Ltd. SM: Researcher, Dimerix Bioscience Pty Ltd. Nothing to Disclose: HBS, RMS, MAA

Pub # P1-179
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - G Protein-Coupled Receptor Biology (1:30 PM-3:30 PM)
Title Investigation into Orexin Receptor- β -Arrestin Signaling Complexes
Author String WC Jaeger, KA Eidne, KDG Pflieger
Western Australian Institute for Medical Research & Centre for Medical Research, University of Western Australia, Nedlands, Australia

Body Orexin receptors 1 and 2 (OxR1, OxR2) play key roles in the metabolic orchestration between energy regulation and sleep/wake cycles. Both receptors display agonist-dependent binding to both β -arrestins, however, OxR2 has been observed to form more stable interactions while OxR1 forms more transient interactions as detected by bioluminescence resonance energy transfer (BRET) (1). In this study, the composition and contribution of the C-terminal tail to OxR- β -arrestin-binding was explored using BRET in HEK293 cells by constructing an OxR1/OxR2 C-tail chimera. This did not confer the expected increase in stability of β -arrestin interaction, but instead resulted in a near-complete ablation of β -arrestin-binding. Inositol phosphate turnover rates were similar for the chimera as for the WT receptor indicating that the Gq-mediated signaling component of the chimeric construct had not been compromised. Subsequently, mutant OxR2 constructs containing alanine substitutions in serine/threonine cluster sites associated with GRK phosphorylation and β -arrestin-binding (2) were investigated using BRET. The data indicates redundancy in the requirement of these sites for β -arrestin-binding. This suggests that although primary structure of the C-terminal tail is important for determining the apparent affinity of receptor and β -arrestin, secondary and perhaps tertiary structure is also likely to be important. MAPK p44/42 (ERK1/2) signaling mediated by OxA stimulation of both OxRs was investigated using a homogeneous cell-based assay. Temporal differences in the level of ERK1/2 phosphorylation (pERK1/2) of both OxRs between 10 and 120 min post-agonist stimulation were observed. Additionally, dose-response data of OxA-stimulated pERK1/2 by OxR2 exhibited a significant reduction in potency after 90 min compared to 2 min post-stimulation. These data are consistent with a β -arrestin-mediated component that is temporally distinct from typical G-protein mediated pERK1/2. Our data indicate that temporal differences in pERK1/2 signaling due to agonist activation of OxR1 compared to OxR2 could relate to subtle differences in β -arrestin binding kinetics. This study provides evidence that the nature of the agonist-induced β -arrestin-receptor complex may be responsible for subtype-specific signaling differences, a better understanding of which may help unlock the therapeutic potential of these receptors.

- (1) Pflieger et al., *Front Neuroendocrinol* 2006; 27:82
(2) Marchese et al., *Annu Rev Pharmacol Toxicol* 2008; 48:601

Sources of Research Support: National Health and Medical Research Council (NHMRC) of Australia via Project Grant 404087 awarded to KAE and KDGP, and Fellowships 212064 and 353709 awarded to KAE and KDGP respectively. WCJ is the recipient of an Australian Postgraduate Award and KDGP is now an Australian Research Council Future Fellow (FT100100271).

Disclosures: KDGP: Chief Scientific Officer, Dimerix Bioscience Pty Ltd. Nothing to Disclose: WCJ, KAE

Pub # P1-180

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Connexin 43 Controls Osteocyte Viability and Osteocytic Gene Expression in a Cell Autonomous Fashion and Regulates Endocortical Resorption and Periosteal Bone Formation

Author String N Bivi, K Condon, JD Benson, N Farlow, G Passeri, T Bellido, LI Plotkin
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Body Connexin 43 (Cx43) mediates osteocyte communication with other cells and with the extracellular milieu and regulates osteoblastic cell signaling and gene expression. We now report that mice lacking Cx43 in osteoblast and osteocytes (Cx43^{[Delta]Ob-Ot^{-/-}}) or only in osteocytes (Cx43^{[Delta]OT} mice) exhibit increased osteocyte apoptosis in cortical bone, elevated endocortical resorption and increased periosteal bone formation, resulting in higher marrow cavity and total tissue areas. Blockade of resorption reversed the increase in marrow cavity but not in total tissue area, demonstrating that endocortical resorption and periosteal apposition are independently regulated. Anatomical mapping of apoptotic osteocytes, bone surfaces undergoing resorption and formation, and osteocytic gene expression, revealed that Cx43 controls osteoclast and osteoblast activity by modulating osteoprotegerin and sclerostin expression, respectively, in osteocytes located in specific areas of the cortex. Moreover, whereas empty lacunae and living osteocytes lacking osteoprotegerin were distributed throughout cortical bone in Cx43^{[Delta]OT} mice, apoptotic osteocytes and osteoclasts were preferentially located in the posterior portion of the cortex, demonstrating that osteoclast recruitment requires active signaling from dying osteocytes. We conclude that Cx43 is essential in a cell autonomous fashion for osteocyte survival and for controlling the expression of osteocytic genes that affect osteoclast and osteoblast function.

Nothing to Disclose: NB, KC, JDB, NF, GP, TB, LIP

Pub # P1-181

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title The Rho-Type Guanine Nucleotide Exchange Factor AKAP13 Is Required for Normal Bone Formation and Expression of Runx2

Author String H Koide, XC Guo, D Donahue, PH Driggers, T Kino, A DeCherney, JH Segars
Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD; National Institutes of Health, Bethesda, MD; Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD; Uniformed Services University of the Health Sciences, Bethesda, MD

Body
Background: The Protein Kinase A Anchoring Protein 13 (AKAP13 or a.k.a. Brx) is the guanine nucleotide-exchange factor for Rho-type small GTP-binding (G), and functions as a cytoplasmic integrator or docking platform for multiple signaling cascades including those of the protein kinase A and nuclear hormone receptors, such as estrogen and glucocorticoid receptors. All these signaling cascades are important for bone metabolism, while Rho-type small G proteins are essential for mediating mechanical stress, an important stimulator of bone formation. Thus, we examined involvement of AKAP13 in the regulation of bone development by employing AKAP13 haploinsufficient mice and MC3T3-E1 cells, an established model of osteoblastic cells.
Materials and Methods: Femur radiographs of seven 20 week-old *AKAP13* +/- mice were visualized with microcomputed tomography scanning and 3-D images were subsequently reconstructed by the volume rendering method using CT-analyzer program. Total RNA was extracted from femurs of the wild type (WT) and *AKAP13* (+/-) mice, and transcript levels were quantified by real-time RT-PCR. MC3T3-E1 cells were differentiated with treatment of beta glycerophosphate and L-ascorbic acid, as reported previously. MC3T3-E1 cells were transfected with AKAP13 or control siRNA to knockdown AKAP13 mRNA.
Results: Bone mineral density, bone volume/total volume and trabecular numbers of the distal portion of femurs were significantly reduced in *AKAP13* +/- mice, compared to WT mice (reductions of 10.9%, 14.0% and 12.8%, respectively). Trabecular spacing was also increased (14.7%) significantly in *AKAP13* +/- mice, resembling the changes observed in osteoporotic bones. mRNA levels of Runx2 and alkaline phosphatase (ALP), an essential differentiation factor for osteogenic lineage and an indicator of osteoblast formation, respectively, were both reduced in femurs of *AKAP13* +/- mice. Knockdown of *AKAP13* reduced ALP mRNA levels and enzymatic activity in proliferating MC3T3-E1 cells, while decreased levels of ALP and Runx2 transcripts in differentiated MC3T3-E1 cells.
Conclusion: AKAP13 plays an essential role in the bone formation/development possibly by stimulating expression of Runx2 and its downstream effector molecules including ALP. Our results suggest that AKAP13 might integrate signal inputs from mechanical stress mediated by small G proteins and action of steroid hormones to Runx2-mediated bone formation.

Sources of Research Support: Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD.

Nothing to Disclose: HK, XCG, DD, PHD, TK, AD, JHS

Pub # P1-182

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Stabilization of Beta-Catenin in *mTert*-Expressing Cells Results in Depletion of Mesenchymal Stem Cells and Bone Loss

Author String DL Carlone, RD Riba, DM Ambruzs, JE Sagers, DT Breault
Children's Hospital Boston, Boston, MA; Harvard Medical School, Boston, MA

Body Proper development, remodeling and regeneration of bone are dependent on the activity of multipotent mesenchymal stem cells (MSCs). Although these cells can be enriched *in vitro*, they are characteristically heterogeneous. Establishing the tools to identify and study these cells will greatly advance our understanding of their role in bone biology, which will lead to improved treatment options for bone diseases. Telomerase (*Tert*) activity prevents cellular senescence and is required for maintenance of stem cells in regenerative tissues. We have generated transgenic mouse lines (*mTert*-GFP and *mTert*-rtTA) that allow for the identification, isolation and genetic manipulation of *mTert*-expressing cells. Previously, we showed that *mTert*-GFP expression marks telomerase-expressing ES cells, iPS cells and self-renewing tissue stem cells. Based on these prior studies we hypothesize that *mTert* expression is a biomarker for MSCs. To investigate whether *mTert* expression marks MSCs, we isolated GFP+ and GFP-ve cells from the non-hematopoietic fraction of bone marrow using flow cytometry and assayed their ability to form fibroblastic-colony forming units (CFU-Fs), a characteristic of MSCs. Fifteen percent of GFP+ cells gave rise to colonies compared with only 0.5% of GFP-ve cells. These results demonstrate that *mTert*-GFP+ cells exhibited a 30-fold enrichment in their capacity to form CFU-Fs and indicate that nearly all of the mesenchymal stem cell activity is found within the GFP+ cell population. The Wnt/ β -catenin signaling pathway plays an important role in bone homeostasis and has been shown to enhance stem cell self-renewal. To investigate the role of Wnt signaling in *mTert*+ cells, we stabilized β -catenin (resulting in constitutive activation) specifically within *mTert*-expressing cells and their subsequent progeny. Intriguingly, stabilization of Wnt/ β -catenin signaling within this population resulted in a short-term increase (2-3 fold) and long-term depletion (~80%) of CFU-Fs, suggesting an exhaustion of the MSC population. In addition, initial studies indicate that long-term stabilization of Wnt/ β -catenin signaling results in decreased osteoblast differentiation *in vitro* and a corresponding decrease in cortical and trabecular bone *in vivo*. These studies indicate that *mTert* expression is a biomarker for MSCs and that Wnt signaling regulates MSC self-renewal and function. Further analysis of these cells may translate into new therapeutics for bone abnormalities.

Sources of Research Support: NIH Grant DK084056 to DTB; Harvard Stem Cell Institute award to DTB.

Nothing to Disclose: DLC, RDR, DMA, JES, DTB

Pub # P1-183

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title The Cytoskeletal Regulatory Scaffold Protein GIT2 Modulates Osteoblastogenesis

Author String X Wang, S Liao, ER Nelson, R Schmalzigaug, RF Spurney, F Guilak, RT Premont, D Gesty-Palmer
Duke University Medical Center, Durham, NC; Duke University Medical Center, Durham, NC; Duke
University Medical Center, Durham, NC

Body G protein-coupled receptor kinase interactor 2 (GIT2) is a signaling scaffold protein involved in the regulation of cytoskeletal structure, membrane trafficking, and G protein-coupled receptor internalization. Since dynamic cytoskeletal reorganization plays key roles both in osteoblast differentiation and in the maintenance of osteoclast polarity during bone resorption, we hypothesized that skeletal physiology would be altered in GIT2^{-/-} mice. We find that adult GIT2^{-/-} mice have decreased bone mineral density and bone volume in both the trabecular and cortical compartments. This osteopenia is associated with decreased numbers of mature osteoblasts, diminished osteoblastic activity, and increased marrow adiposity, suggesting a defect in osteoblast maturation. In vitro, bone marrow cells derived from GIT2^{-/-} mice exhibit impaired differentiation into osteoblasts and increased adipocyte differentiation, consistent with a role for GIT2 in mesenchymal stem cell fate determination. Despite elevated osteoclast inducing cytokines and osteoclast numbers, GIT2^{-/-} mice also have impaired bone resorption, consistent with a further role for GIT2 in regulating osteoclast function. Collectively, these findings underscore the importance of the cytoskeleton in both osteoblast and osteoclast function and demonstrate that GIT2 plays essential roles in skeletal metabolism, affecting both bone formation and bone resorption in vivo.

Sources of Research Support: The Arthritis Foundation (DGP).

Nothing to Disclose: XW, SL, ERN, RS, RFS, FG, RTP, DG-P

Pub # P1-184

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Strontium Ranelate Prevents Advanced Glycation End Product[en dash]Induced Deleterious Effects on Osteoblastic Cells: Role of Pro-Inflammatory Cytokines

Author String C Sedlinsky, JM Fernandez, MS Molinuevo, L Schurman, AM Cortizo, AD McCarthy
Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina

Body The formation and accumulation of advanced glycation end products (AGE) have been implicated in the pathogenesis of several metabolic disorders, including diabetes. In patients suffering from diabetes type 2 it is frequent to find alterations in bone tissue, possibly associated to an accumulation of AGE on bone tissue. We have previously demonstrated that AGE causes a deleterious effect on bone related cells. The mechanisms underlying these effects include induction of osteoblastic apoptosis, increase in reactive oxygen species as well as a decrease in osteoblastic differentiation. It has also been demonstrated that the interaction of AGE with its specific receptor RAGE induces NFk-B activation that in turn increases pro-inflammatory cytokines secretion such as interleukin 1- β (IL-1 β) and TNF- α . In the present work we evaluated the action of Strontium Ranelate (SR) on the deleterious action of AGE on osteoblasts. MC3T3E1 osteoblastic line was cultured in the presence of different concentrations of glycated bovine serum albumin (AGE) or unmodified-albumin (BSA) as control, with or without strontium ranelate. After 24h incubation, 0.1 mM of SR increased cell proliferation (122 \pm 5 % of BSA) while AGE inhibited cell proliferation in a dose dependent manner, being the maximum effect at 200 [μ]g/ml AGE (63 \pm 4 % of BSA, p<0.01). AGE also inhibited alkaline phosphatase activity (100 [μ]g/ml AGE: 60 \pm 8 % vs BSA, p<0.01) and collagen production (100 [μ]g/ml AGE: 79 \pm 4 % vs BSA, p<0.01) after 15 days of culture. These effects of AGE were completely prevented by co-incubation with SR. Evaluation of cytokines production showed that SR tends to decrease secretion of both IL-1 β and TNF- α while AGE increased IL 1- β and TNF- α secretion (twofold over control BSA, p<0.01). AGE-induced cytokine secretion was prevented by 0.1 mM of SR co-incubation. In order to elucidate the role of calcium channels on the action of SR, MC3T3E1 osteoblasts were incubated for 24h with nifedipine, a type L calcium channel blocker. Nifedipine was able to inhibit the anabolic action of SR on osteoblastic cells as well as the effect of SR on AGE action. In conclusion, we have shown that SR could inhibit the deleterious action of AGE on osteoblastic proliferation and differentiation as well as proinflammatory cytokine secretion. We also demonstrated that SR action depends on the activation of calcium channels to exert its action.

Nothing to Disclose: CS, JMF, MSM, LS, AMC, ADM

Pub # P1-185

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title The Role of Nuclear Factor-E2-Related Factor 1 in Oxidative Stress Response in Osteoblast Cells

Author String SY Park, SM Choi, SH Lee, SJ Park, S Lim
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Body
Objective : Nuclear factor-E2-related factor 1 (Nrf1, NFE2L1) is known to regulate the expression of a number of genes involved in oxidative stress and inflammation. The purpose of this study was to examine the effects of Nrf1 on the response to oxidative stress in osteoblastic cells. This study also examined the effects of Nrf1 on osteoblastic proliferation under unstimulated and oxidative stress conditions.
Material and Methods : The murine calvaria-derived MC3T3E1 cell line was exposed to lipopolysaccharide (LPS) for oxidative stress induction. Nrf1 effects were evaluated using siRNA for Nrf1 mRNA. Reactive oxygen species (ROS) generation and the levels of known antioxidant enzyme genes were assayed. In addition, cell proliferation analysis was performed. Statistical analysis was performed using Student' t-test or one-way analysis of variance (ANOVA) with Tukey's post hoc test ($p < 0.05$).
Results : Nrf1 expression was significantly increased 2.4-fold compared to the control group at 10 μ g/ml LPS in MC3T3E1 cells ($p < 0.05$). LPS increased formation of intracellular ROS in MC3T3E1 cells. Nrf1 knockdown led to an additional increase of ROS (20%) in the group transfected with Nrf1 siRNA compared with the control group under LPS stimulation ($p < 0.05$). RNA interference of Nrf1 suppressed the expression of antioxidant genes including metallothionein 2 (MT2), glutamate-cysteine ligase catalytic subunit (GCLC) and glutathione peroxidase 1 (GPx1) in LPS-treated MC3T3E1 cells. Knockdown of Nrf1 expression did not affect MC3T3E1 cell proliferation under unstimulated or oxidative stress conditions.
Conclusion : Our results suggest that Nrf1 may have a distinct role in the regulation of antioxidant enzymes under inflammation-induced oxidative stress in osteoblasts.

Nothing to Disclose: SYP, SMC, SHL, SJP, SL

Pub # P1-186

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Globular Adiponectin Increases the Phosphorylation of AKT, ERK1/2 and CREB in MC3T3-E1 Cells, Affecting Their Proliferation, Gene Expression and Glucose Uptake

Author String M Berrou, B Taha, A Fite, NS Datta, AB Abou-Samra
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Body Adiponectin is an adipocyte-derived hormone with a wide range of biological effects. In contrast to its function in insulin sensitization, adiponectin's role in bone biology is still a topic of controversy. This study examines the effects of globular adiponectin's (gAdp) on key-player molecules in osteoblast physiology. The MC3T3-E1 subclone 4 (MC4), a widely accepted cell line model for studying osteoblasts, was treated with gAdp alone, and/or in presence of insulin or parathyroid hormone (PTH). Briefly, gAdp increased the phosphorylation of AKT[S474], p44/42 MAPK (ERK1/2) [Thr202/Tyr204] and cAMP response element-binding protein CREB[S133] in a dose and time-dependent manner. Pretreatment of the cells with Wortmannin, a potent PI3K inhibitor, resulted in complete inhibition of gAdp's induced AKT phosphorylation. On the other hand pretreatment with U0126, a MEK (MAP/ERK kinase) inhibitor, completely inhibited ERK1/2 phosphorylation. Co-treatment with hPTH(1-34) (100 nM) significantly decreased gAdp-AKT[S474] and ERK1/2 [Thr202/Tyr204] phosphorylation. Insulin addition, on the other hand, didn't change gAdp's effect on these molecules. As AKT, ERK1/2 and CREB are involved in cell proliferation; we evaluated the effects of gAdp on MC4 proliferation using the MTT colorimetric assay. Treatment of MC4 cells with gAdp significantly increased the number of viable cells by 16% after 24 hr of treatment and increased the mRNA expression of the following genes: Adipor1, Adipor2, Insr, PTH1R, Glut1, Glut3, Glut4, Runx2 and c-FOS. Treatment with gAdp also increased glucose uptake at 30 min and at 24 h. While the increased glucose uptake at 30 min may be explained by Glut4 translocation, the further significant increase at 24 h suggests an increase in protein transcription. Insulin-stimulated glucose uptake didn't change with gAdp treatment. In summary, our study links globular adiponectin with multiple aspects of osteoblast biology and shows crosstalk with PTH signaling. Globular Adp may regulate osteoblast differentiation, proliferation and PTH signaling; this highlights the complexity of bone and adipose tissue interactions.

Sources of Research Support: NIH/NIDDK and institutional funding.

Nothing to Disclose: MB, BT, AF, NSD, ABA-S

Pub # P1-187

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title PPAR Activity Modulates the Interaction of Bone Morphogenetic Proteins and Tumor Necrosis Factor- α in Osteoblast Differentiation

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Impaired functions and balance of osteoclasts and osteoblasts are critical for the progression of inflammatory bone diseases. Clinical effectiveness of TNF- α inhibition for treating rheumatoid arthritis has established the significant roles of TNF- α in the pathogenesis of inflammatory bone damages. Recent clinical and basic studies have also suggested the possible adverse effects of thiazolidinediones on the bone formation. However, the detailed mechanism by which PPAR actions are involved in the bone metabolism has been controversial. Here we investigated the cellular mechanism by which PPAR activities interact with osteoblast differentiation regulated by BMP and TNF- α using mouse myoblastic C2C12 cells. Among the BMP ligands, BMP-2 and -4 potently induced the expression levels of bone differentiation markers including Runx2, osteocalcin and collagen-1 and alkaline phosphatase in C2C12 cells. Notably, BMP-4 actions in combination with PPAR α agonist (fenofibric acid) but not PPAR γ agonist (pioglitazone) most potently enhanced osteoblast differentiation. The osteoblastic changes induced by BMP-4 were readily suppressed by treatment with TNF- α . Interestingly, PPAR α and PPAR γ agonists reversed the TNF- α suppression of osteoblastic differentiation induced by BMP-4 with restoring TNF- α -induced suppression of BMP signaling. Furthermore, TNF- α -induced phosphorylation of MAPKs, including ERK, p38-MAPK and SAPK/JNK, NF[κ]B, I[κ]B and Stat pathways was inhibited in the presence of PPAR α and PPAR γ agonists with reducing the expression levels of TNF type-1 and -2 receptors. Given the finding that chemical inhibitions of SAPK/JNK, Stat and NF[κ]B pathways reversed the TNF- α suppression of osteoblastic differentiation, these cascades are functionally involved in the actions of PPARs that antagonize TNF- α -induced suppression of osteoblast differentiation. It is further revealed that PPAR α agonist, but not PPAR γ agonist, enhanced BMP-4-induced Smad phosphorylation and BMP target gene Id-1 transcription through downregulating Smad6/7 expression. On the other hand, BMPs exhibited increasing effects on the expression levels of PPAR α/γ in C2C12 cells. Thus, PPAR α actions promote BMP-induced osteoblast differentiation, while both activities of PPAR α and PPAR γ suppress TNF- α actions. Collectively, PPAR activities are functionally involved in modulating the interaction between the BMP system and TNF- α receptor signaling that are crucial for the bone metabolism.

Nothing to Disclose: MT, FO, YM, KI, MT, EN, NT, TM, K-ES, HM

Pub # P1-188

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title The Effects of Estrogen Receptor A and B Specific Agonists and Antagonists on Cell Proliferation and Energy Metabolism in a Human Bone Cell Line

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Body We have reported that in cultured human female bone cells estradiol-17 β (E2) modulated DNA synthesis, the specific activity of creatine kinase BB (CK), 12 and 15 lipoxygenase (LO) mRNA expression and formation of 12- and 15- hydroxyeicosatetraenoic acid (HETE), the arachidonic acid derived metabolites of these enzymes. We now investigate the response of human bone cell line (SaOS2) to estrogen receptors specific agonists and antagonists. Treatment of SaOS2 with E2, 2,3-bis (4-hydroxyphenyl)-propionitrile (DPN; ER β specific agonist) and 4,4',4''-[4-propyl-(1H)-pyrazol-1,3,5-triyl] tris-phenol (PPT; ER α specific agonist) showed increased DNA synthesis and stimulated CK. Raloxifene (Ral), an ER α antagonist, inhibited E2 or PPT stimulations, but not DPN. The other ER α specific antagonist methyl-piperidino-pyrazole (MPP) and the ER β specific antagonist 4-[2-Phenyl- 5, 7-bis (tri-fluoro-methyl) pyrazolo [1, 5-a] pyrimidin-3-yl] phenol (PTHPP) inhibited specifically DNA synthesis, CK and reactive oxygen species (ROS) formation induced by estrogenic compound. The LO inhibitor baicaleine did not affect PPT but inhibited E2 and DPN effects. E2 had no effect on ER α mRNA expression whereas DPN and PPT stimulated them. ER β mRNA expression was stimulated by all compounds. All estrogenic compounds modulated the expression of 12 and 15LO mRNA and 12 and 15 HETE productions. All hormones stimulated ROS formation which was inhibited by NADPH oxidase inhibitor diphenylene iodonium chloride (DPI). DPI did not significantly affect hormonal induced cell proliferation and energy metabolism. In conclusion, we provide herein evidence for the separation of mediation via ER α and ER β pathways in the effects of E2 on osteoblasts, but the exact mechanisms and the role of ROS are unclear.

Nothing to Disclose: DS, SK, OS, MG-C, EK, NS

Pub # P1-189

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Effect of Thiazolidinediones on Osteoblast Biomarkers

Author String P Suwandhi, AS Pareek, A Seth, VV Sy, N Branis, S-Y Kim, L Poretsky, D Seto-Young
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Body

Introduction: Thiazolidinediones [TZDs, rosiglitazone (R) and pioglitazone (P)] are insulin sensitizers used for treatment of diabetes. Previously, we reported that TZDs inhibit osteoblast cell growth and decrease alkaline phosphatase (AP) activity (1). In this report, we describe the effects of TZDs on osteocalcin (OC), osteoprotegerin (OPG), and procollagen mRNA expression in mouse osteoblast cell (MOC) cultures and MOC/human granulosa cell (HGC) co-cultures. MOC/HGC co-cultures were used to determine whether TZD inhibition of aromatase (CYP19) may play a role in bone metabolism.

Materials & Methods: MOCs were co-cultured with and without HGC and incubated in a medium with or without testosterone (T), P, or R. AP activity, OC and OPG production, along with pro-collagen mRNA expression was examined. AP activity was measured by spectrophotometry at 450 nm; OC was measured by RIA; OPG was measured by ELISA; and pro-collagen mRNA expression was determined by RT-PCR.

Results: TZD (5-25[μ M]) inhibition of AP activity in MOC or MOC/HGC co-cultures was dose-dependent. For MOC and MOC/HGC cultures, AP was inhibited by 60% to 70% ($p < 0.005$) similarly in the absence or presence of T. TZD effect on OC exhibited a dose-related response, inhibiting OC production up to 60% ($p < 0.05$) in both cultures similarly in the presence or absence of T. In the MOC/HGC co-culture, TZD (10-25 [μ M]) inhibited OPG production. The inhibitory effect of TZDs on OPG was 60-70% ($p < 0.001$) in the absence of T and 50% to 60% ($p < 0.001$) in the presence of T. The studies of the dose effect of TZDs on OPG production are in progress for both MOC and MOC/HGC cultures. Mouse pro-collagen mRNA expression was also inhibited up to 50% by TZDs.

Conclusion: Our data indicate that TZDs significantly inhibit osteoblast biomarkers (AP, OC, OPG) in MOC cultures and MOC/HGC co-cultures in the presence or absence of T. Therefore, TZDs directly affect osteoblast biomarkers independently from their CYP19 effect. TZD inhibition of OPG production suggests that TZDs induce osteoclast activity. These data are consistent with clinical trials (2-4). Studies of TZD effects on RANKL are in progress.

(1) Seth A et al., Thiazolidinediones (TZDs) Inhibit Bone Turnover. Endocrine Society: The 92nd Annual Meeting & Expo. 2010;P2-184.
(2) Okazaki R et al., Endocrine Journal 1999;46(6):795-801.
(3) Berberoglu Z et al., J Clin Endocrinol Metab 2007;92:3523-3530.
(4) Zinman B et al., J Clin Endocrinol Metab 2010;95(1):134-142.

Sources of Research Support: Gerald J. and Dorothy Friedman New York Foundation for Medical Research; Empire Clinical Research Investigator Program of the New York State Department of Health; Chinese American Medical Society and Chinese American Independent Practice Association; Yen Family Foundation.

Nothing to Disclose: PS, ASP, AS, VVS, NB, S-YK, LP, DS-Y

Pub # P1-190

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Dissociation of Bone Resorption and Bone Formation in Adult Mice with a Non-Functional V-ATPase in Osteoclasts Leads to Increased Bone Strength

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Body Osteopetrosis caused by defective acid secretion by the osteoclast, is characterized by defective bone resorption, increased osteoclast numbers, while bone formation is normal or increased. In contrast the bones are of poor quality, despite this uncoupling of formation from resorption.
To shed light on the effect of uncoupling in adult mice with respect to bone quality, we transplanted irradiated three-month old normal mice with fetal liver cells from control or oc/oc mice, which have defective acid secretion, and followed them for 12 to 28 weeks.
Engraftment levels were assessed by flow cytometry of peripheral blood. Serum samples were collected every six weeks for measurement of bone turnover markers. At termination bones were collected for ^{45}Ca CT and mechanical testing.
An engraftment level of 98% was obtained. From week 6 until termination bone resorption was significantly reduced, while the osteoclast number was increased when comparing oc/oc to controls. Bone formation was elevated at week 6, normalized at week 12, and reduced onwards. ^{45}Ca CT and mechanical analyses of femurs and vertebrae showed increased bone volume and bone strength of cortical and trabecular bone.
In conclusion, these data show that attenuation of acid secretion in adult mice leads to uncoupling and improves bone strength.

Sources of Research Support: The Danish Research Foundation.

Disclosures: MAK: Employee, Nordic Bioscience A/S. Nothing to Disclose: KH, CF, JST, A-MB, CST, AVN-W, GEJL, NAS, MA, TJM, VE, JR

Pub # P1-191

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Pycnogenol, a French Maritime Pine Extract, Suppresses RANKL-Stimulated Osteoclast Differentiation of RAW264.7 Cells

Author String H Watanabe, B Fan, S Ikuyama
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Body Pycnogenol is a natural compound extracted from the bark of French maritime pine. It is widely used as a dietary supplement or traditional medicine which exerts strong antioxidant effects. One of its possible effects is relief of climacteric symptoms in postmenopausal women. It is, therefore, particularly of interest whether Pycnogenol is useful for the prevention of postmenopausal osteoporosis. To confirm this presumption, we tested the effects of Pycnogenol on receptor activator of NFκB ligand (RANKL)-induced osteoclast differentiation. When RAW264.7 cells were incubated in the presence of RANKL (12.5-50 ng/ml) for 5 days, the expression of tartrate-resistant acid phosphatase 5 (TRAP5) mRNA significantly increased. Pycnogenol (12.5-50 mg/ml) significantly suppressed RANKL-induced TRAP5 expression in a dose-dependent manner. Pycnogenol also suppressed RANKL-stimulated activity of the TRAP5b promoter activity. In the presence of Pycnogenol, RANKL-induced osteoclast differentiation, as evidenced by TRAP-positive multinuclear cell formation, was also significantly suppressed. Since AP-1 and NFκB are important transcription factors involved in RANKL-induced osteoclast differentiation, we tested the effects of Pycnogenol on enhancer activity and DNA binding of these factors in RAW264.7 cells. Contrary to our expectation, RANKL-induced AP-1 or NFκB enhancer activity was not suppressed by Pycnogenol. In addition, RANKL-induced activation of DNA binding of these factors was not suppressed by Pycnogenol. These results suggest that Pycnogenol acts at the site after activation of these transcription factors involved in the osteoclast differentiation pathway. Whatever the underlining molecular mechanism is, Pycnogenol might be a promising agent for suppression of excessive bone resorption.

Sources of Research Support: Pycnogenol was provided by Horphag Research (Switzerland).

Nothing to Disclose: HW, BF, SI

Pub # P1-192

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Interleukin-6 Receptor Signaling Plays a Role in Anabolic Actions of PTH in Bone

Author String SW Cho, FQ Pirih, MN Michalski, M Eber, SJ Oh, TJ Wronski, LK McCauley
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Body Intermittent administration of parathyroid hormone (PTH) increases bone mass by stimulating bone formation. Interleukin (IL)-6 is upregulated by PTH and we previously reported that IL-6 is a critical mediator of PTH effects on hematopoietic cells. The purpose of this study was to determine the dependence of PTH on IL-6 for skeletal anabolic actions of PTH. Intermittent PTH (iPTH, 50 mg/kg/day) was injected to: a) young (3d) wildtype (WT) and IL-6 deficient (IL-6 KO) mice for 3wks and, b) adult (16wk) WT and IL-6 KO mice for 6wks. Bone histomorphometry of tibia and vertebrae and microCT analysis of femurs showed that WT and IL-6 KO mice had a similar increase in bone area with PTH in both young and adult mice. Dynamic histomorphometry of adult mice showed no difference in bone formation rate and mineral apposition rate between WT and IL-6 KO mice treated with iPTH. To investigate potential compensatory mechanisms, protein levels of soluble IL-6 receptor (sIL-6r) were examined. iPTH administration was performed in 16wk WT and IL-6 KO mice for 2 weeks, and sIL-6r was measured in serum and bone marrow by ELISA. In basal conditions, serum sIL-6r was significantly reduced in IL-6 KO mice compared with WT mice, however sIL-6r in bone marrow was similar between WT and IL-6 KO mice. Interestingly, treatment with iPTH significantly increased bone marrow sIL-6r in both WT and IL-6 KO mice. The IL-6 signaling pathway was blocked using soluble glycoprotein 130 receptor (sgp130r). Young IL-6 KO mice were treated with: 1) vehicle (VEH), 2) iPTH (50 mg/kg/day) only (PTH), and 3) PTH and sgp130r (250ng/day) (PTH + sgp130r) and bone histomorphometry performed 2 wks later. The PTH group had increased bone volume and trabecular number which was significantly attenuated when sgp130r was combined with PTH. Consistent with histomorphometric parameters, serum levels of P1NP were also significantly decreased in PTH + sgp130r group compared to PTH group. Collectively, IL-6 KO mice demonstrated a normal anabolic response to iPTH administration. Protein levels of sIL-6r were preserved and similarly increased by iPTH treatment in bone marrow of IL-6 KO mice versus WT mice. Treatment with a specific inhibitor of sIL-6r to IL-6 KO mice significantly attenuated the anabolic action of iPTH. This study suggests that IL-6 receptor signaling is critical for anabolic actions of iPTH in bone.

Sources of Research Support: NIH DK53904 awarded to L.K.M.

Nothing to Disclose: SWC, FQP, MNM, ME, SJO, TJW, LKM

Pub # P1-193

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title *De Novo* 3-Kb *STX16* Deletions Leading to Pseudohypoparathyroidism Type Ib

Author String S Turan, J Ignatius, J Moilanen, H Stewart, M Bastepe, H Juppner
Massachusetts General Hospital and Harvard Medical School, Boston, MA; Marmara University, Istanbul, Turkey; Turku University Hospital, University of Turku, Turku, Finland; Oulu University Hospital, University of Oulu, Oulu, Finland; Oxford Radcliffe Hospitals NHS Trust, Oxford, UK; Massachusetts General Hospital and Harvard Medical School, Boston, MA

Body Pseudohypoparathyroidism (PHP) is characterized by hypocalcemia, hyperphosphatemia, and elevated PTH levels due to end-organ resistance to this hormone. Patients with PHP type Ib (PHP-Ib) typically exhibit isolated renal resistance to PTH and lack features of Albright's osteodystrophy (AHO). A maternally inherited 3-kb deletion within *STX16* (encoding syntaxin-16) is the most common cause of autosomal dominant PHP-Ib (AD-PHP-Ib), characterized by isolated loss of methylation at the A/B differentially methylated region of *GNAS*. Here we described a sporadic case of PHP-Ib and a multi-generational AD-PHP-Ib kindred in whom *de novo* 3-kb *STX16* deletions were identified.

The sporadic PHP-Ib case presented with convulsions at the age of 9.5 years due to hypocalcemia (1.55 mmol/L) with elevated PTH (403 pg/ml) and phosphorus (2.56 mmol/L). There was no evidence for AHO and no family history for PHP. Methylation analysis revealed isolated loss of A/B methylation, consistent with AD-PHP-Ib. Analysis of genomic DNA revealed the 3-kb *STX16* deletion in the patient, but not in his healthy mother, father, or sister. Microsatellite analysis of the *GNAS* region showed that the patient shared the same maternal allele with his unaffected sister. The mother was heterozygous for marker 261P9-CA1 (located within the *STX16* region), while the patient revealed evidence for allelic loss, indicating that the identified mutation had occurred *de novo* on the maternal allele. In second family, the index case presented with low calcium (1.41 mmol/L) and elevated PTH (381 pg/ml) at the age of 8 years without evidence for AHO. He has five siblings, three of whom also showed biochemical features of PTH resistance. Genetic analysis revealed the 3-kb *STX16* deletion in all affected siblings, as well as their healthy mother and a healthy maternal uncle. Analysis of other extended family members revealed no evidence for the 3-kb deletion, but haplotype analysis of the *GNAS* region suggested that the deceased maternal grandfather of the index case had been carrier of a *de novo* *STX16* deletion. Since he had no history of hypocalcemia, it is likely that the mutation had occurred on his paternal allele. *De novo* 3-kb *STX16* deletions appear to be rare events since only 3 such mutations (including the two reported herein) have been reported among the more than 30 reported families with AD-PHP-Ib.

Sources of Research Support: Research grants from National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases (R01 DK073911 to MB and R37 DK46718 to HJ).

Nothing to Disclose: ST, JI, JM, HS, MB, HJ

Pub # P1-194

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title A Model of Hyperparathyroidism in Female Mice: Effects of Ovariectomy on Bone Mass and Microarchitecture, Serum Minerals, PTH Secretion and Gene Expression

Author String D Shoback, T-H Chen, N Liang, M You, Z Cheng, C Tu, W Chang
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Body Heterozygous deletion of the calcium-sensing receptor (CaSR) targeted to the parathyroid (PT-CaSR^{+/-}) produces mild hypercalcemia and elevated PTH in mice. The bone phenotype and abnormalities in PTH secretion, however, show striking age- and gender-dependence. Female PT-CaSR^{+/-} have higher plasma PTH levels than wild-type (wt) control and male PT-CaSR^{+/-} mice and a marked shift to the right in the Ca set-point for secretion in vitro vs wt females. Trabecular (Tb) bone parameters by micro-CT indicate an osteopenic phenotype in PT-CaSR^{+/-} females vs preserved bone mass in PT-CaSR^{+/-} males. Estrogen (E) regulates mineral homeostasis through effects on bone, PT glands, and other tissues. We assessed serum biochemistries and micro-CT parameters; Ca-regulated PTH secretion in vitro in PT glands; and gut mRNA by qPCR using 12-wk-old wt and PT-CaSR^{+/-} mice post ovariectomy (OVX) or sham surgery (SHAM). Serum [Ca] was mildly elevated in PT-CaSR^{+/-} (10.1±0.2 mg/dL) vs wt mice (9.7±0.2 mg/dL) (N=11; p<0.05) and did not change with OVX. PTH levels, in contrast, rose from 215±45 in wt-SHAM to 364±80 pg/mL in wt-OVX (N=10, p=0.06) and to greater levels from 407±81 (PT-CaSR^{+/-} SHAM) to 709±74 pg/mL (PT-CaSR^{+/-} OVX) (N=8, p<0.01). Tb bone parameters were similarly affected by OVX in wt and PT-CaSR^{+/-} mice with significantly decreased bone volume/tissue volume (BV/TV) and Tb number and thickness and increased Tb spacing (vs SHAM mice). The Ca set-point (extracellular [Ca] required for half-maximal suppression of PTH) was shifted to the right from 1.12±0.03 mM in wt glands to 1.32±0.04 mM in PT-CaSR^{+/-} in PT-CaSR^{+/-} glands (N=6-8, p<0.001). In contrast, the post-OVX Ca set-point was left-shifted in glands from PT-CaSR^{+/-} (1.08±0.04 mM) vs PT-CaSR^{+/-} post-SHAM (1.32±0.04 mM) (p<0.001) supporting a direct effect of loss of E on PTH, operating independent of Ca-sensing. PCR on gut RNA from female PT-CaSR^{+/-} showed increased vitamin D receptor (~50%), CaSR (~300%), and TRPV6 channel (~50%) expression vs wt and decreased CaSR and TRPV6 mRNA post-OVX. In contrast to mild primary hyperparathyroidism (HPT) in postmenopausal women, in whom Tb bone mass is often stable over time, loss of E due to OVX in mice with mild HPT promotes bone loss, raises PTH levels, alters PT cell sensitivity to [Ca] and affects expression of genes key to gut Ca transport. This model will enable the testing of molecules involved in interactions between PTH, sex steroids, and target organs.

Sources of Research Support: Research Service, Department of Veterans Affairs; National Institutes of Health, NIAMS and NIA; Department of Defense.

Nothing to Disclose: DS, T-HC, NL, MY, ZC, CT, WC

Pub # P1-195

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Npt2a-NHERF1-Ezrin Ternary Complex Mediates PTH-Sensitive Phosphate Transport

Author String B Wang, Y Yang, PA Friedman
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Body The parathyroid hormone type 1 receptor (PTH1R) mediates PTH actions on phosphate homeostasis primarily by regulating the activity of type 2a sodium-phosphate cotransporter (Npt2a) by a mechanism that requires the PDZ protein Na/H Exchanger Regulatory Factor, NHERF1. However, the precise interplay of these proteins is not fully understood. Opossum kidney (OK) cells express endogenous PTH1R, NHERF1 and Npt2a proteins, and display a robust PTH-mediated inhibition of inorganic phosphate (Pi) uptake. The OKH cell subclone expresses minimal NHERF1. By using the OKH cells, we found that wild-type NHERF1 interacted with Npt2a, but not with Npt2a harboring a mutant PDZ-binding sequence. NHERF1 lacking the ezrin-binding domain does not interact with Npt2a. PTH induced the dissociation of wild-type NHERF1 from Npt2a in OK cells. We also found that PTH applied to the basolateral cell surface of OKH cells transfected with wild-type NHERF1 cells and grown on permeable filter supports decreased the apical membrane Npt2a abundance. NHERF1 interacts with Npt2a and ezrin, a protein that bridges NHERF1 with the actin cytoskeleton. We measured the assembly of a ternary complex of Npt2a, NHERF1, and ezrin by sequential Co-IP of HA-Npt2a, elution with excess HA peptide followed by IP of FLAG-NHERF1, and subsequent detection of ezrin by immunoblotting. Unlike wild-type NHERF1, the formation of the Npt2a-NHERF1-ezrin ternary complex by mutated Npt2a and truncated NHERF1 constructs was significantly inhibited. Finally, we analyzed the functional role of this ternary complex in mediating PTH-sensitive Pi transport. St-Ht3, which disrupts the interaction between ezrin and the type II regulatory subunit of PKA (RII), inhibited PTH-sensitive Pi uptake by OKH cells transfected with wild-type NHERF1 but had no effect on basal levels of Pi uptake. We conclude that PTH regulates renal Pi transport by a cAMP-PKA pathway that involves the formation of a ternary complex of Npt2a, NHERF1, and ezrin. We propose that ezrin serves as a dual-function A-kinase anchoring protein (AKAP). These results suggest additional pathophysiological mechanisms that may be responsible for causing PTH resistance and PTH1R downregulation.

Sources of Research Support: NIH R01 DK069998.

Nothing to Disclose: BW, YY, PAF

Pub # P1-196

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title PTH1R Conformational Dynamics upon Occupancy by Non-Activating Ligands

Author String JA Ardura, J-P Vilardaga, PA Friedman
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Body The type I parathyroid hormone receptor (PTH1R) regulates extracellular calcium and phosphate homeostasis. PTH1R prominently expressed in bone and kidney promotes activation of adenylyl cyclase and phospholipase C in response to biologically active forms of PTH, PTH(1-34) and PTH(1-84). Although PTH(1-84) is the major secreted form of PTH, other PTH fragments that are likely to be PTH(7-84) are also generated. These PTH peptides or their synthetic analogs (PTH[7-34]) are thought to be inactive because, despite binding to the receptor, they fail to stimulate cAMP and calcium. However, these amino-truncated PTH peptides efficiently internalize and downregulate the PTH1R by mechanisms that are not fully understood. Here, we report differences in PTH1R conformational dynamics induced by PTH(1-34) compared to PTH(7-84). We used a Fluorescence Resonance Energy Transfer (FRET)-based cAMP biosensor and the calcium sensitive dye Fluo-4 to analyze signaling by live-cell microscopy of rat osteosarcoma cells. PTH(1-34) increased cAMP production and accumulation of intracellular calcium. In contrast, PTH(7-84) failed to trigger cAMP or calcium signaling. Pretreatment of cells with PTH(7-84) blocked cAMP and calcium signaling induced by subsequent exposure to PTH(1-34). This inhibition was not due to PTH1R internalization because blocking receptor endocytosis with dominant-negative dynamin failed to restore PTHR signaling. We used an engineered FRET-based PTH1R biosensor to characterize the conformational changes in response to PTH(1-34) and PTH(7-34). PTH(1-34) induced a rapid conformational shift ($t_{1/2}$ = 1-2 sec), whereas PTH(7-34) produced a significantly slower ($t_{1/2}$ = 15 sec) change of PTH1R conformation. We conclude that inactivating PTH ligands induce distinct receptor conformations that trigger PTH1R internalization without stimulating G protein activation of adenylyl cyclase and phospholipase C.

Sources of Research Support: NIH R01DK 054171.

Nothing to Disclose: JAA, J-PV, PAF

Pub # P1-197

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title HRPT2 Mutation in Parathyroid Carcinoma and Atypical Parathyroid Adenoma

Author String S Niramitmahapanya, T Sunthornthepvarakul, C Deerochanawong, V Sarinnapakorn, P Athipan, P Harnsomboonvana, S Kongsangdao
Rajavithi Hospital, Medical College of Rangsit University, Bangkok, Thailand; Rajavithi Hospital, Medical College of Rangsit University, Bangkok, Thailand; Medical Collage of Rangsit University, Bangkok, Thailand

Body **Objective:** Parathyroid carcinoma is an uncommon endocrine malignancy. It is the least common endocrine malignancy with a prevalence of 0.005% (1) of all cancers. The purpose of this study was to detected HRPT2 genes mutation in parathyroid carcinoma. (2) and atypical parathyroid adenoma in sporadic hyperparathyroidism of Thai patients. HRPT2 gene mutation can be a helpful tool in expanding understanding of rare diseases.

Methods: Samples of parathyroid carcinoma, adenoma or hyperplasia were obtained for HRPT2 gene (17 exons) study since September 2001 to August 2010 both somatic and germline by SyBr Green PCR method.

Results: Parathyroid carcinomas and atypical parathyroid adenoma from five of 26 patients (10 of 32 samples) were tested for HRPT2 mutations. Only somatic HRPT2 mutations were found in tumor from two patients but three patients found both somatic and germline HRPT2 mutations. Exon15 of HRPT2 gene was the best sensitivity (sensitivity 80%; $p < 0.001$, 95%CI; 0.75-1.05) followed by exon2 and exon11 (sensitivity 60%; $p = 0.007$, 95%CI; 0.60-0.99) to detected parathyroid carcinoma or atypical parathyroid adenoma.

Conclusion: HRPT2 gene mutation can be a helpful tool in expanding understanding of rare diseases. Exon15 of HRPT2 gene mutation was the most common followed by exon2 and exon11 in parathyroid carcinoma or atypical parathyroid adenoma. Genotyping of such family members for germline mutation would focused implementation of clinical and biochemical monitoring of carriers of these mutations.

(1) Hundahl S, Fleming I, Fremgen A, et al. Two hundred eighty-six cases of parathyroid carcinoma treated in the US between 1985-1995, a national cancer data base report. *Cancer* 1999;86:538-44.

(2) Shattuck TM, Valimaki S, Obara T, et al. Somatic and germ-line mutations of the HRPT2 gene in sporadic parathyroid carcinoma. *N Engl J Med.* 2003; 349:1722-1729.

Nothing to Disclose: SN, TS, CD, VS, PA, PH, SK

Pub # P1-198

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Preclinical PK and PD Relationship for KAI-4169, a Novel Calcimimetic

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Body KAI-4169 is a novel peptide calcimimetic for the treatment of secondary hyperparathyroidism (SHPT) in patients with end-stage renal disease (ESRD). In numerous preclinical studies in rats and dogs, treatment with KAI-4169 has been shown to reduce parathyroid hormone (PTH) levels in the blood consistent with its ability to activate the calcium sensing receptor (CaSR). Here we describe the pharmacokinetics (PK) and pharmacodynamics (PD) of KAI-4169 following intravenous (IV) administration in preclinical models of normal and compromised renal function. The PK studies conducted demonstrate the predictable and consistent PK of KAI-4169 in rats (CLobs 240-271 mL/hr/kg; Vssobs 448-517 mL/kg; t_{1/2} ~1.3 hr) and dogs (CLobs 363 - 409 mL/hr/kg; Vssobs 1710-1990; t_{1/2} 6.2 - 7 hr). A comparison of parameters in rats with normal and compromised renal function highlights the importance of renal function in the clearance of KAI-4169 as evidenced by a three-fold reduction in clearance in the absence of renal function. KAI-4169 administration results in a dose-dependent reduction in PTH levels, both in terms of magnitude and duration of effect. These data advance our understanding of the relationship between the PK and PD effect of KAI-4169. A clear exposure-response relationship was demonstrated that was found to be consistent within a given species. The mode of administration did not have a clear impact on the threshold plasma concentration of KAI-4169 required to reduce PTH levels; similar EC₅₀ values were obtained after IV bolus and infusion administration. Preclinical PK/PD data were used to perform allometric projections of human PK and human equivalent dose: of KAI-4169 in both normal, healthy human subjects as well as subjects with compromised renal function (e.g., ESRD patients). The predicted PK parameters along with PD information obtained from animal models informed the estimation of appropriate doses for Phase I trials and permitted the prediction of likely concentration-time profiles after IV dosing in humans prior to initiating Phase I trials.

Nothing to Disclose: SW, AV, TH, AB, JJ, SA, DM

Pub # P1-199

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Paricalcitol Treatment Decreases Atherosclerosis in APOE Null Mice: A Pilot Study

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Body Epidemiologic evidence suggests vitamin D is inversely correlated with blood pressure and with cardiovascular morbidity and mortality. We previously demonstrated that a low dose of calcitriol (0.25 ng/g body weight) decreases atherosclerosis in APO E^{-/-} mice by down regulation of renin, the rate-limiting step of the renin-angiotensin system. At the dose used there was no apparent effect on immunologic processes (T-regs, inflammatory cytokines). However, even at this relatively low dose, prolonged calcitriol administration is limited by ensuing hypercalcemia. Less calcemic analogs are in clinical use in hemodialysis subjects, and have shown clinical benefit with respect to cardiovascular mortality. In a parallel preliminary study, chronic treatment of Tsukuba Hypertensive Mice with low dose paricalcitol didn't induce any rise in serum calcium. We therefore sought to investigate the effect of paricalcitol on the development of atherosclerosis in APO E^{-/-} mice.

Methods: At 7 weeks, ApoE null mice were switched to an atherogenic diet. 5 animals received paricalcitol as an intraperitoneal injection of 0.25 ng/g body weight every other day for 8 weeks, while control mice (n=6) received the vehicle only. The extent of atherosclerosis at the aortic sinus was assessed by quantification of Oil-Red-O-stained lesions. Biochemical parameters were also assessed at the end of the study.

Results: Paricalcitol reduced the extent of atherosclerosis at the aortic sinus by 60% (P=0.005). Paricalcitol treatment had no significant effect on any of the metabolic parameters: glucose, cholesterol, triglycerides. Additionally, paricalcitol treatment had no effect on the weight of the mice nor on the weight of their hearts relative to their body weight.

Summary and Conclusions: In this pilot study, paricalcitol treatment had a significant anti-atherogenic effect in ApoE null mice. A larger study with escalating doses likely to have a direct impact on immunologic mechanisms involved in atherogenesis, will help determine the optimal regimen.

Nothing to Disclose: MI-S, MV, NS, KMT

Pub # P1-200

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Vitamin D Metabolites and Synthetic Less-Calcemic Vitamin D Analogs Induce Reactive Oxygen Species (ROS) Formation as a Signal to Inhibit Human Arterial Vascular Smooth Muscle Cell Proliferation

Author String D Somjen, M Grafi-Cohen, O Sharon, E Knoll, A Many, N Stern
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Body Although most vitamin D's actions are traditionally ascribed to 1, 25(OH)₂D₃ [1,25D] acting on classical vitamin D receptors, there is now evidence that 24, 25 (OH)₂D₃ [24,25D], formerly considered merely an inactivation product, has important independent biological effects as well. Synthetic less-calcemic vitamin D analogs are presumed to act through classical vitamin D receptors but induce lesser rise in serum calcium *in vivo*. Here we examined the effects of these various vitamin D receptor modulators on ROS in arterial vascular smooth muscle (VSMC) harvested from the human umbilical artery, in the context of their known modulatory effects on VSMC proliferation as reported by us in earlier communications (Am. J. Hypertens. 2000; 13:396; J. Steroid Biochem. Mol. Biol. 2004; 89-90:397; Circulation 2005; 111:1666). With the exception of very low concentrations, [1,25D], [24,25D], and 25 (OH)D₃ [25D] and the less calcemic synthetic analogs JKF and QW, all decreased VSMC proliferation by 30-60% along with parallel increments in cell metabolic activity as reflected by the ATP-generating system creatine kinase BB (CK). These vitamin-D related agents also increased ROS formation as examined by direct visualization in a fluorescent microscopy system. There were differences in the induction of ROS, which was minimal with [25D] and [1, 25D], potent with [24,25D] and extremely potent with JKF and QW. When the formation of ROS was blocked by diphenylene iodonium (DPI), an inhibitor of NADPH oxidase, the effect of all vitamin D-related compounds on ROS formation was entirely aborted. Likewise, in the presence of DPI, none of the vitamin D-related compounds was able to inhibit VSMC proliferation and CK induction was also attenuated. These results establish a link between the inhibitory effect of vitamin D metabolites and analogs on VSMC growth and ROS formation. ROS formation apparently serves to allow the transduction of vitamin-D induced signals aimed at slowing down VSMC proliferation. This is an energy requiring process which is also blocked when ROS generation is inhibited. These events in the vasculature must be further studied especially in times in which liberal use of mega-doses of vitamin D in clinical medicine is highly fashionable.

Nothing to Disclose: DS, MG-C, OS, EK, AM, NS

Pub # P1-201

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title A Low Dose of the Less-Calcemic Vitamin D Analog Paricalcitol Lowers Blood Pressure in Tsukuba Hypertensive Mice (THM) by Affecting Renin Expression without Causing Hypercalcemia or Hypercalciuria

Author String KM Tordjman, M Vechoropoulos, L Rona, M Ish Shalom, N Stern
Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

Body

Introduction: Vitamin D has lately gained much interest for its potential cardiovascular protective effects. We recently showed that vitamin D suppresses renin expression, lowers blood pressure, and reduces atherosclerosis in the Tsukuba Hypertensive Mouse (THM), a model of hypertension and atherosclerosis due to the transgenic expression of the human renin-angiotensin system (RAS). However, even at low dose, the prolonged duration of treatment required for the anti-atherogenic effect resulted in hypercalcemia and an unfavorable metabolic profile, a potentially significant limitation to clinical application. We sought to investigate the effect of a similar low dose of paricalcitol (**P**) on blood pressure and metabolic parameters in THM.

Methods: At age 8-10 weeks, THM animals were allocated to either **P**, given IP at a dose of 0.25 ng.g⁻¹ every other day (n=22), or to the vehicle-**C** (n=17) for 4 weeks. In the final week of the experiment, animals were studied in metabolic cages. In addition to serum biochemistry, plasma renin activity (PRA) was measured by RIA. Expression of both the endogenous and the human transgenic RAS components (renin, AGT, AT1-R and ACE) was assessed by real-time PCR.

Results: Baseline systolic BP was 137±2.9 mmHg. By the end of the study it had decreased to 116.2±3.8 in **P** ($P=0.0005$), while it rose to 147.1±3.4 in **C** ($P=0.04$ vs baseline and $P<0.0001$ compared to **P**). **P**-treated mice drank significantly less than **C** (5.6±0.4 vs. 7.0±0.3 ml.20 g⁻¹.d⁻¹, $P=0.01$). **P** treatment had no effect on any of the biochemical parameters. Serum calcium was 8.8±0.4 mg/dl in **P** and 9.2±0.3 in **C**. Likewise, urinary calcium excretion was unaffected and was 488±24 [μ]g.mouse⁻¹.d⁻¹ in **P** and 440±45 in **C**. Despite significant variability, PRA showed a definite trend toward lower values with **P**, 275±170 vs 428±164 ng.ml⁻¹.h⁻¹ in **C**, $P=0.06$. By real-time PCR, **P** caused a 79% reduction in the human renin mRNA level at the aorta ($P=0.04$), while a 51% reduction in the mouse renin mRNA didn't reach significance. None of the other genes assessed showed any significant alteration.

Conclusions: In THM, paricalcitol treatment for 4 weeks was as efficient as calcitriol in reducing BP, but in contrast it caused no derangement in calcium metabolism. If extended to the anti-atherogenic effect of calcitriol previously demonstrated in this model, clinical studies to assess its efficacy in the treatment of hypertension and the prevention of atherosclerosis might be warranted.

Nothing to Disclose: KMT, MV, LR, MIS, NS

Pub # P1-202

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Vitamin D Supplementation Improves Cognitive Function and Alters Hippocampal Gene Expression in Aging Rats

Author String CS Latimer, LD Brewer, EM Blalock, PW Landfield, NM Porter
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Body There is growing concern regarding the extent of Vitamin D (VitD) deficiency in the general population, but especially in the elderly who are at greatest risk. Despite the prevalence of VitD deficiency in the elderly, relatively little is known about how VitD affects the brain and cognitive function. Based on our prior in vitro and in vivo studies, we suspect that increased VitD levels may reverse markers of brain aging and counteract, at least some, aspects of age-related brain decline (Brewer et al., 2001; 2006). Because this age-related brain decline begins to appear around midlife, this age may represent a critical window of opportunity for the manipulation of VitD status.

In order to test the hypothesis that age-related brain decline is slowed or prevented by higher VitD levels, dietary manipulation of VitD was initiated at midlife and continued for 4-5 months. Male rats (12 month old) were divided into three groups and fed diets containing varying amounts of VitD: low, normal (conventional), or high. Following chronic treatment, a memory-based water maze task was used to provide potential insight into how VitD status may affect age related brain decline. This task required the rats to find a hidden platform in a pool of water using visual cues placed around the pool. Additional microarray studies were performed to identify potential gene pathways targeted by VitD.

Our studies indicate that vitamin D status affects cognitive function during aging. We found that maintaining higher levels of vitamin D during middle-age appears to be an important factor in preserving and extending healthy brain function and cognitive ability. Specifically, middle-aged rats maintained on the high VitD diet were more successful in the memory-based task than the normal and low VitD fed rats. Additionally, microarray analysis revealed selective expression of genes involved in synaptic function in the hippocampus of animals fed the high VitD diet. This alteration in hippocampal gene expression may represent an underlying mechanism by which VitD appears to improve the likelihood of successful brain aging.

(1)Brewer et al., J Neurosci.21:98-108, 2001
(2)Brewer et al., Cell Calcium 40(3):277-86, 2006

Sources of Research Support: NIH AG10836; NIH AG000242.

Nothing to Disclose: CSL, LDB, EMB, PWL, NMP

Pub # P1-203

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Vitamin D Sufficiency Is Associated with an Anti-Inflammatory Monocyte Phenotype

Author String A Riek, J Oh, M Petty, C Bernal-Mizrachi
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Body Macrophages and their monocyte precursors are a critical determinant for the development of vascular complications in type 2 diabetes mellitus (type 2 DM). Macrophage subtypes (pro-inflammatory M1 and anti-inflammatory M2) are believed to influence the evolution of inflammation in the vascular wall, but there is a lack of knowledge of the role of the different monocyte phenotypes in the early stages of vascular inflammation. In patients with type 2 DM, the prevalence of 25-hydroxy vitamin D deficiency is almost twice that for non-diabetics, and low vitamin D levels nearly double the risk of developing cardiovascular disease or nephropathy compared to diabetic patients with normal vitamin D levels. We tested the hypothesis that monocytes from vitamin D deficient type 2 DM subjects will have a pro-inflammatory phenotype compared to those from vitamin D sufficient diabetics. We obtained monocytes from vitamin D deficient (n=13) or sufficient (n=10) type 2 DM patients. Surprisingly, monocytes from vitamin D sufficient diabetics exhibited a 15% increase in the ratio of M1/M2 macrophage phenotype markers (membrane receptor expression of CCR7 plus CD86 divided by CD163 plus mannose receptor) when compared to monocytes obtained from vitamin D deficient individuals (p<0.03). However, vitamin D sufficient monocytes adhered 49-70% less to both human umbilical vein endothelial cells or fibronectin at different time points from 30 minutes to 16 hours compared to vitamin D deficient monocytes (p<0.0005) and had a ~33% decrease in monocyte migration induced by MCP1 compared to vitamin D deficient monocytes (p<0.02). Vitamin D sufficient monocytes had significantly lower mRNA expression of the adhesion markers beta-1 integrin, beta-2 integrin, and e-selectin when compared to monocytes obtained from vitamin D deficient individuals (p<0.04 for all receptors). Thus, vitamin D sufficiency was associated with an anti-inflammatory monocyte phenotype, and macrophage phenotypic markers may not represent the same functionality in monocytes.

Sources of Research Support: AER is supported by the NIH/NCRR Washington University CTSA grant UL1 RR024992.

Nothing to Disclose: AR, JO, MP, CB-M

Pub # P1-204

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Vitamin D Metabolism in Human Bone Marrow Stromal Cells

Author String MS LeBoff, S Zhou, S Geng, J Glowacki
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Body Low vitamin D levels have been associated with reduced bone strength, skeletal fragility, and fractures. We recently found expression and activity of CYP27B1/1 α -hydroxylase (CYP27B1) in human bone marrow stromal cells (hMSCs) that was influenced by the vitamin D status of the subjects from whom the cells were obtained.¹ In this study, we tested the hypothesis that vitamin D metabolism in hMSCs is involved in their differentiation to osteoblasts. Discarded femoral heads were obtained from 49 consented subjects (age 41-83 years) whose serum 25OHD levels (Diasorin RIA) were obtained. Adherent hMSCs from low-density marrow mononuclear cells were expanded in α -MEM medium with 10% FBS-HI. Constitutive gene expression was evaluated by semi-quantitative RT-PCR. The 1,25(OH)₂D levels secreted in media were quantitatively determined with enzyme immunoassay. Alkaline phosphatase activity was measured colorimetrically 7 days after cultured in osteogenic supplements. First, osteoblastogenesis *in vitro* was stimulated to a greater degree by 1,25(OH)₂D₃ in hMSCs obtained from subjects who were D-deficient (25OHD levels <20 ng/ml) than those who were not (p<0.05); thus, repletion of vitamin D-deficient subjects may lead to more vigorous bone formation. Second, hMSCs expressed the various vitamin D hydroxylases (CYP27B1, CYP27A1, CYP2R1, CYP24A1) and the vitamin D receptor. There was equivalent stimulation of osteoblastogenesis in hMSCs by 1,25(OH)₂D₃ (10 nM), 25OHD₃ (100 nM), or vitamin D₃ (1000 nM); this suggests that hMSCs may synthesize 1,25(OH)₂D₃ from 25OHD₃ and vitamin D₃. Third, hMSCs (from a 42-year-old man) produced ~0.30 nM 1,25(OH)₂D₃ from added 25OHD₃, which is in the stimulatory dose range for 1,25(OH)₂D₃ (0.01 to 10 nM). Fourth, the *in vitro* hydroxylation of 25OHD to 1,25(OH)₂D and the stimulation of osteoblastogenesis by 25OHD₃ were blocked by ketoconazole, a cytochrome P450 inhibitor, and by CYP27B1-siRNA; thus, the activation of 25OHD₃ to 1,25(OH)₂D₃ by CYP27B1 is necessary for the biological effects of 25OHD₃. Finally, CYP24A1 was upregulated in hMSCs by 1 nM 1,25(OH)₂D₃ and by 10 nM 25OHD₃; thus, hMSCs can regulate the local concentration of 1,25(OH)₂D₃ by its rates of production and inactivation. In sum, this study provides evidence that vitamin D metabolism in hMSCs may have an autocrine/paracrine role in osteoblast differentiation and may be regulated locally and systemically for maintenance of bone health.

(1) Zhou S, LeBoff MS, Glowacki J. *Endocrinology* 2010;151:14.

Nothing to Disclose: MSL, SZ, SG, JG

Pub # P1-205

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Effects on AMPK Activation in a Breast Cancer Cell Model by 1,25D₃

Author String LE Tavera-Mendoza, T Westerling, M Brown
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Body The active form of vitamin D, 1,25-dihydroxyvitamin D₃ (1,25D₃), regulates gene expression through the vitamin D receptor (VDR), which is member of the nuclear receptor superfamily. Even though 1,25D₃ is still best known as a regulator of bodily calcium homeostasis, 1,25D₃ has a plethora of physiological actions which include immune and nervous system modulation and cellular proliferation and differentiation. Epidemiological and model studies have revealed the cancer-protective properties of 1,25D₃ in colon, prostate and breast. In this study, we investigated the anti-proliferative properties of 1,25D₃ in a panel of human breast cancer cell lines. We found that 1,25D₃ induces autophagy in several luminal breast cancer cell models, but not in basal or mesenchymal models. We also found that 1,25D₃ induced autophagy requires p27 stabilization through 1,25D₃ mediated skp2 inhibition and AMPK activation. We performed both VDR and RXR ChIP-seq analysis and uncovered several 1,25D₃ target genes involved in regulation of metabolism. Our studies suggest that energy regulation is a novel aspect of 1,25D₃ anti-proliferative properties in breast cancer cells.

Sources of Research Support: DOD Fellowship W81XWH-09-1-0570 awarded to LETM.

Nothing to Disclose: LET-M, TW, MB

Pub # P1-206

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Production of 22-Hydroxy Metabolites of Vitamin D₃ by Cytochrome P450scc (CYP11A1) and Analysis of Their Biological Activities on Keratinocytes

Author String RC Tuckey, W Li, H Shehabi, Z Janjetovic, MN Nguyen, T-K Kim, DE Howell, J Chen, T Sweatman, DD Miller, AT Slominski

Body The University of Western Australia, Crawley, Australia; University of Tennessee Health Science Center, Memphis, TN; Curtin University of Technology, Perth, Australia; University of Tennessee Health Science Center, Memphis, TN; University of Tennessee, Health Science Center, Memphis, TN

Cytochrome P450scc (CYP11A1) can hydroxylate vitamin D₃ to 20S-hydroxyvitamin D₃ (20(OH)D₃) and 20S,23-dihydroxyvitamin D₃ (20,23(OH)₂D₃) as the major products. Minor products include, 17-hydroxyvitamin D₃, 17,20-dihydroxyvitamin D₃ and 17,20,23-trihydroxyvitamin D₃. The major products exhibit potent biological activity on skin cells including inhibiting proliferation and NF-κB activity and stimulating differentiation, in a similar fashion to 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), but unlike 1,25(OH)₂D₃ they are poor inducers of CYP24 expression. Furthermore, they are non-calcemic at doses up to 3 [micro]g/kg in rodents and therefore may be useful therapeutically. In the current study we have analysed the reaction products from P450scc action on vitamin D₃ using different HPLC conditions and have identified two 22-hydroxy-derivatives of vitamin D₃ as minor products, 22-hydroxyvitamin D₃ (22(OH)D₃) and 20,22-dihydroxyvitamin D₃ (20,22(OH)₂D₃). The structures of both of these derivatives were determined by NMR. We compared the biological activities of these new enzymatically produced derivatives to those of 20(OH)D₃ and 20,23(OH)₂D₃, as well as to 25(OH)D₃ and 1,25(OH)₂D₃ controls. Only 1,25(OH)₂D₃, 20(OH)D₃ and 20,23(OH)₂D₃ significantly (by ANOVA) inhibited keratinocyte proliferation at 48 and 72 h of treatment in a dose-dependent manner. Although 22(OH)D₃, 20,22(OH)₂D₃ and 25(OH)D₃ show a trend to inhibit proliferation after 72 h it was not significant by ANOVA (p>0.05). The strongest inducers of keratinocyte differentiation, analysed using involucrin immunofluorescence as a marker, were 20(OH)D₃, 20,22(OH)₂D₃, 20,23(OH)₂D₃ and 1,25(OH)₂D₃, with 22(OH)D₃ having little effect. 20(OH)D₃, 22(OH)D₃, 20,22(OH)₂D₃ and 20,23(OH)₂D₃ all significantly stimulated mRNA expression for the vitamin D receptor, but not to the extent seen for 1,25(OH)₂D₃. Little or no stimulation of CYP24 mRNA expression was observed for all the analogues tested except for 1,25(OH)₂D₃ which caused the expected strong stimulation. In contrast, all the compounds stimulated VDR translocation from the cytoplasm to the nucleus with 22(OH)D₃ showing the lowest potency. Thus we have identified 22-hydroxyderivatives of vitamin D₃ as products of CYP11A1 action on vitamin D₃ and shown that like 20(OH)D₃ and 20,23(OH)₂D₃, they are active on keratinocytes working via the VDR, with 22(OH)D₃ generally showing weaker effects than the other P450scc-derived hydroxymetabolites of vitamin D₃.

Sources of Research Support: R01AR052190 from NIH/NAIMS.

Nothing to Disclose: RCT, WL, HS, ZJ, MNN, T-KK, DEH, JC, TS, DDM, ATS

Pub # P1-207

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Vitamin D Status in Obesity Is Associated with Skeletal Muscle Mass Independent of Adiposity

Author String P Shantavasinkul, P Phanachet, O Puchaiwattananon, L-o Chailurkit, B Ongphiphadhanakul, D Warodomwichit
 Ramathibodi Hospital, Faculty of Medicine, Mahidol University, Bangkok, Thailand; Ramathibodi Hospital, Faculty of Medicine, Mahidol University, Bangkok, Thailand; Ramathibodi Hospital, Faculty of Medicine, Mahidol University, Bangkok, Thailand

Body Vitamin D deficiency is now being recognized as emerging problems worldwide. Obesity has been found to be associated with lower level of serum 25-hydroxyvitamin D (25(OH) D) due to decreased bioavailability from sequestration of vitamin D in fat compartments. This cross-sectional study was designed to assess the prevalence of vitamin D deficiency and the relationship between serum 25(OH) D and body composition in healthy obese subjects. This study was conducted in Bangkok, the capital city of Thailand, which is situated near the equator (the latitude of 13°45'N). The average duration of sunlight in Thailand is 5 to 9 hours per day. We recruited 162 Thai obese subjects (38.3% were male). The mean (\pm SD) of age and body mass index (BMI) were 41.3 \pm 11.8 years and 33.6 \pm 6.6 kg/m², respectively. Overall, the mean (\pm SD) level of 25(OH)D was 23.02 \pm 5.22 ng/ml. The level of 25(OH)D was significantly higher in men (24.33 \pm 5.53 vs. 22.20 \pm 4.86 ng/ml, $P=0.01$). In our study, 48/162 (29.6%) and 148/162 (91.4%) of obese individual had vitamin D deficiency (25(OH)D <20 ng/ml) and vitamin D inadequacy (25(OH)D <30 ng/ml), respectively. All obese subjects with BMI \geq 35 kg/m² had vitamin D inadequacy. The level of 25(OH)D were negatively associated with percent body fat ($r = -0.24$, $P=0.002$). Moreover, vitamin D level was positively associated with skeletal muscle mass ($r = 0.17$, $P=0.03$) and the association was exist after controlling for body fat mass and age ($P=0.003$). Male subjects had significantly more skeletal muscle mass (mean \pm SD; 36.1 \pm 5.8 kg vs. 24.7 \pm 3.5 kg, $P < 0.001$) and less percent body fat (mean \pm SD; 35.1 \pm 8.4 vs. 44.8 \pm 5.3, $P < 0.001$) compared with female subjects. Higher fat mass and lower skeletal muscle mass were correlated with lower 25(OH) D levels which may attribute to the higher prevalence of vitamin D deficiency in women (34% vs. 22.6%). Our study demonstrated not only the high prevalence of vitamin D deficiency in obese population even they live near the equator but also the association between vitamin D status and skeletal muscle mass independent of adiposity. The benefit of vitamin D supplement to the body composition and insulin sensitivity in the obese subjects is now being assessed in the ongoing trial.

1. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;72:690-3.

Sources of Research Support: Thailand Research Fund; Faculty of Medicine, Ramathibodi Hospital, Mahidol University.

Nothing to Disclose: PS, PP, OP, L-OC, BO, DW

Pub # P1-208

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Calcidiol and Calcitriol Ameliorate Statin-Induced Myopathy in C2C12 Myotubules

Author String AW Gannon, T Ventura-Holman, J Subauste
University of Mississippi/GV Sonny Montgomery Veterans Affairs Medical Center, Jackson, MS; University of Mississippi, Jackson, MS

Body Background: Statins are the most important class of cholesterol-lowering medications and are generally well tolerated, however they are recognized to cause muscle cell injury in some patients. Patients with low serum calcidiol levels are particularly susceptible to this myopathy and normalization of these calcidiol levels results in improved statin tolerance in the majority of these patients (1), however it is unclear whether calcidiol or calcitriol is the effector of this change or the mechanism by which it is achieved. There is no lab model of statin myopathy where calcidiol or calcitriol have been demonstrated to reduce muscle cell injury. C2C12 myotubules have been demonstrated to be an effective model of statin-induced myopathy (2) and so we sought to develop a model for the effects of Vitamin D analogs in amelioration of statin-induced myopathy. Design: C2C12 myoblasts were differentiated into myotubules using Dubecco's Modified Eagle's Medium with 2% Horse Serum and once fully differentiated they were incubated in stripped serum with either calcitriol 10nM, calcidiol 100nM or calcidiol 250nM for 4 days and then exposed to mevinolin 1[μ]M and observed for 4 days. The medium was refreshed every 48 hours throughout the course and photographs were taken immediately before mevinolin exposure and then again 2 days and 4 days later. Cells were harvested at each step for biochemical evaluation. The ImageJ software package (NIH) was utilized to measure myotubule diameter. Results: C2C12 myotubules that were exposed to calcitriol and either concentration of calcidiol without mevinolin did not demonstrate any significant phenotypic difference compared to control. Myotubules deprived of Vitamin D and exposed to mevinolin were severely injured with approximately 60% reduction in diameter. Myotubules preincubated in calcitriol were relatively spared with only 20% reduction in diameter and preservation of many large-diameter tubules. Similarly, myotubules exposed to low and high doses of calcidiol demonstrated approximately 20% reduction in myotubule diameter compared to vitamin D deprived cells and had preservation of larger myotubules. Further biochemical analysis with RT-PCR and Western blot are planned. Conclusion: C2C12 myotubules exposed to either calcidiol or calcitriol are relatively protected from mevinolin-induced myopathy. Further studies into the mechanisms of this protection are in order to better understand this phenomenon.

(1) Ahmed W et al. Trans Research, 153 (1): 11-16.

(2) Hanai J et al., J Clin Investigation, 117 (12): 3940-3951

Nothing to Disclose: AWG, TV-H, JS

Pub # P1-209

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Local Activation of Vitamin D Increases Adipogenesis in Human Subcutaneous Preadipocytes

Author String H Nimitphong, M-J Lee, SK Fried, MF Holick
Boston University School of Medicine, Boston, MA

Body The extrarenal activation of vitamin D (VD) modulates its actions in many tissues. Human fat stores VD and is a target tissue for VD. We found that human adipose tissues as well as preadipocytes expressed all of the enzymes necessary to activate VD [25-hydroxylases (CYP2R1 and CYP27A1) and 1 α -hydroxylase (CYP27B1)] as well as the vitamin D receptor (VDR). We cultured human preadipocytes with 1,25(OH)₂D₃ (10⁻⁸ or 10⁻¹⁰ M), 25(OH)D₃ (10⁻⁸ or 10⁻⁹ M) or vitamin D₃ (10⁻⁶ M). Addition of vitamin D₃ or 1,25(OH)₂D₃ and to a lesser extent, 25(OH)D₃, increased CYP24A1 expression, a known VDR target; in newly-differentiated adipocytes, only 25(OH)D₃ and 1,25(OH)₂D₃ were effective. We tested whether VD affected differentiation of human preadipocytes. Addition of 1,25(OH)₂D₃, 25(OH)D₃, and vitamin D₃ to a standard differentiation cocktail (insulin, dexamethasone, IBMX, rosiglitazone) increased the expression of adipogenic markers (LPL and PPAR γ mRNA, FABP4 protein) and triglyceride accumulation (1.5-2-fold). When added after the removal of the differentiation cocktail, 1,25(OH)₂D₃ was equally effective in promoting TG accumulation and expression of adipogenic markers while addition of 1,25(OH)₂D₃ during the first 3d of the differentiation induction period was ineffective, suggesting that 1,25(OH)₂D₃ promotes the maturation stage of adipogenesis. We conclude that the local activation of vitamin D may promote the maturation of human preadipocytes and may play a role in the healthy remodeling of human adipose tissues.

Nothing to Disclose: HN, M-JL, SKF, MFH

Pub # P1-210

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Combinatorial Effects of Nandrolone and Vitamin D₃ on Human Skeletal Muscle Cell Proliferation

Author String HJ Kloosterboer, M Prins, R Class
KC2, Oss, Netherlands; OrgaNext Research, Arnhem, Netherlands; Pharmacelsus, Saarbruecken, Germany

Body
Introduction. Nandrolone decanoate (ND) has been proven to increase muscle mass. We hypothesize that the combination of ND and vitamin D₃ (VitD₃) or NDD lead to an improved stimulation of muscle cell growth and muscle function than ND alone. We studied the effects of the active compounds in human muscle satellite cells.
Materials and methods. Human skeletal muscle cells or satellite cells of a young (hSkMC-1) and an older (hSkMC-2) donor (PromoCell, Germany) were cultured until about 80% confluency in the presence of nandrolone or 1 α , 25-dihydroxyVitD₃. The effect on the androgen (AR) and vitamin D₃ (VD3R) receptors were determined after 5 days. AR and VD3R were measured after double staining with unlabeled primary antibodies and fluorophore labeled secondary antibodies (PE-conjugated for VD3R and FITC-conjugated for AR) followed by flow cytometry measuring the % of stained cells and the staining intensity. The effect on proliferation of both single compounds and the effect of the combination were measured with the resazurin assay after 8 days. Statistical analysis of the data was performed with the Student's t-test.
Results. AR and VD3R are present in both hSkMC-1 and hSkMC-2. In hSkMC-2 the % of cells with positive AR and VD3R staining increases after exposure to nandrolone, as well as the intensity of the staining in cells that were positive before exposure. A similar effect on both the AR and VD3R is seen after exposure to 1 α , 25-hydroxyVitD₃. The effects are less pronounced in hSkMC-1. 100nM and 1000 nM nandrolone or 10nM and 100mM 1 α , 25 hydroxyVitD₃ do not increase proliferation of hSkMC-1 above control. In hSkMC2 cells only the highest concentration of 1 α , 25-hydroxyVitD₃ increases proliferation. Surprisingly the combination of the two compounds, using the same concentrations as with the single compounds, significantly increases proliferation above control in both hSkMC-1 and hSkMC-2.
Conclusions. These in vitro studies demonstrate that: 1) both AR and VD3R are present in satellite cells of human skeletal muscle. 2) nandrolone and 1 α , 25-hydroxyVitD₃ stimulate expression of their cognate and each other receptors in these cells. 3) the response on receptor expression in cells derived from the older dono is stronger than that in the young one and 4) nandrolone and 1 α , 25-hydroxyVitD₃ have a synergistic effect on cell proliferation. Our studies show for the first time a synergistic effect of nandrolone and VitD₃ on human muscle satellite cells.

Disclosures: HJK: Consultant, OrgaNext Research. MP: Owner, OrgaNext Research. Nothing to Disclose: RC

Pub # P1-211

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title New Vitamin D₃ Derivatives: 20-Hydroxy- and 20, 23-Dihydroxyvitamin D₃ Target NF-[kappa]B Receptor in Melanoma Cells

Author String Z Janjetovic, LM Pfeffer, E Escue, AC Wright, RC Tuckey, SR Pfeffer, AT Slominski
University of Tennessee Health Science Center, Memphis, TN; The University of Western Australia, Crawley Australia

Body **Melanoma, the most aggressive form of skin cancer, is highly resistant to current modalities of therapy, with melanogenesis playing an important role in resistance. NF-[kappa]B is constitutively activated in melanoma and can serve as a molecular target for cancer therapy and steroid/secosteroid action. High NF-[kappa]B activity is present in many melanoma cells isolated from different patients with melanoma; observed in this study. Some of them are displaying higher nuclear NF-[kappa]B expression and others lower. Novel vitamin D derivatives 20-hydroxyvitamin D₃ (20(OH)D₃) and 20,23 dihydroxyvitamin D₃ (20,23(OH)₂D₃) and classical 1 α ,25-dihydroxyvitamin D₃, or calcitriol, (1,25(OH)₂D₃) inhibited melanoma cell proliferation, and this effect was seen only in the presence of interferon α (IFN). In summary, high basal NF-[kappa]B activity is seen in most of the tested melanoma cells, pigmented and nonpigmented. In addition, the presence of melanin is associated with the lower inhibition of proliferation of melanoma. Vitamin D derivatives, along with interferon, are a promising treatment for melanoma.**

Sources of Research Support: R01AR052190 from NIH/NAIMS.

Nothing to Disclose: ZJ, LMP, EE, ACW, RCT, SRP, ATS

Pub # P1-212
Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title Different Profiles of Acute PTH Secretion: A Comparison between alpha-Klotho and FGF23 Knockout Mice
Author String A Imura, R Maeda, Y-i Nabeshima
Graduate School of Medicine, Kyoto University, Kyoto, Japan; Japan Science and Technology Corporation, Wako, Japan

Body alpha-Klotho (α -Kl) plays the dual roles in FGF23-signal transduction through capturing the hormonal ligand (Urakawa et al., Nature2006) and the regulated parathyroid hormone (PTH) secretion via Na,K-adenosine triphosphatase(Na,K-ATPase) recruitment (Imura et al., Science2007). The former mechanism suppresses the expression of CYP27B1(1 α -hydroxylase) to downregulate active vitamin D synthesis in hours to days, and the latter one, in response to fluctuations of extracellular calcium concentration, quickly promotes PTH secretion to upregulate tubular calcium reabsorption, bone absorption and vitamin D synthesis in kidney in minutes to hours. Together with these molecular actions, α -Kl achieves chronological maintenance in mineral homeostasis. Since half life of active vitamin D is more than 10 hours, the most crucial phenotype seen in α -Kl-deficiency as well as FGF23-deficiency was hypervitaminosis D due to overexpression of CYP27B1. Consistently, FGF23 transgenic mice revealed a hypovitaminosis D because of CYP27B1 suppression (Shimada T et al., BBRC2004). However, PTH level was significantly low in the mice despite of low vitamin D level. Thereafter, based on the results that FGF23 administration suppressed PTH expression and serum concentration in rats, Ben-Dov IZ et al. discussed that PTH secretion should be affected by FGF23 together with α -Kl (JCI, 2007).
To address whether FGF23 is necessary for PTH regulation, we studied histological features of parathyroid gland and acute profiles of PTH secretion in FGF23-deficient mice. As previously reported, PTH secretion in response to low calcium stimuli was severely impaired in α -Kl-knockout mice (α -Kl-KOM) even at the normalized vitamin D levels using vitamin D-deficient diet (Science, 2007). Interestingly, FGF23-KOM showed the relatively increased amounts of PTH secretion in response to calcium fluctuation compared with those of the litter animals. Thus we considered that FGF23 constitutively suppresses PTH regulation in vivo. The role of FGF23 is contrary to that of α -Kl in parathyroid gland, because α -Kl is required for promoting PTH secretion via Na,K-ATPase translocation. In α -Kl-KOM, therefore, disruption of PTH secretion was dominant rather than unleashed PTH secretion that would be caused by lack of FGF23 signal. PTH downregulation by FGF23 presumably forms a negative feedback loop in mineral homeostasis, where vitamin D level is adjusted at the relevant levels together by α -Kl.

- (1) Imura A et al., Science 2007;316 ;1615
- (2) Urakawa I et al., Nature 2006; 444 ;770
- (3) Shimada T et al., BBRC2004;314;409
- (4) Ben-Dov IZ et al., JCI 2007;117 ;4003

Sources of Research Support: Ministry of Education, Science, and Culture grants 19045016 and 21390058.

Nothing to Disclose: AI, RM, Y-IN

Pub # P1-213

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title α -Klotho Functions as a Glucuronide-Recognizing Protein to Regulate FGF23 Activity Via Binding to O-Linked Glucuronide Moiety

Author String R Maeda, A Imura, Y-i Nabeshima
Kyoto University, Kyoto, Japan; Foundation for Biomedical Research and Innovation, Kobe, Japan; Japan Science and Technology Corporation, Saitama, Japan

Body α -klotho (α -kl) was first identified as an aging gene and later shown to be a regulator of calcium and phosphate homeostasis by binding to Na⁺,K⁺-adenosine triphosphatase (Na⁺,K⁺-ATPase) and FGF23 (Imura A., Maeda R., Nabeshima Y., et al., α -Klotho as a regulator of calcium homeostasis. 2007 Science (1); Tomiyama K., Maeda R., Imura A., Nabeshima Y., et al., Relevant use of Klotho in FGF19 subfamily signaling system in vivo. 2010 PNAS(2)). It has been argued whether α -kl, which is a β -glycosidase homolog acts as an enzyme or as a scaffold protein. However, its precise molecular significance has not been yet cleared. By mass spectrometry, we found a novel O-linked carbohydrate in fibroblast growth factor-23 (FGF23). Since the moieties were first identified in glycans in higher eukaryotes, the evidence was further confirmed by distinct biochemical methods. This O-glycan was found to directly bind to a putative glycosidase domain of α -Kl and facilitate the α -Kl/FGF23 interaction, thereby efficiently trapping circulating FGF23 in the kidney by α -Kl. The α -Kl/FGF23 complex binds to FGF receptor-1 (FGFR1) through the direct interaction of α -Kl and FGFR1, and intra-cellular FGF23 signaling cascades are processed. We propose a novel mechanism of how carbohydrate-binding scaffold proteins and O-glycans of ligands coordinately regulate signal transduction. We also show that a glucuronized-steroid which directly binds to the domain of α -Kl competitively inhibited the interaction of α -Kl and glycosylated FGF23. We discuss (i) how FGF23 signal is transduced via visualizing the ternary α -Kl/FGF23/FGFR1 complex using Atomic Force Microscope (AFM), (ii) a novel molecular mechanism of how α -Kl functions as a carbohydrate-binding protein, and (iii) how tissue specific signaling of FGF23 is achieved by α -Kl. Here we present the data that a glucuronized-steroid inhibited the α -Kl dependent FGF receptor activation as well. The glucuronized-steroid competitively bound to α -Kl with FGF23 and FGFR and consequently impedes to form an active complex of α -Kl, FGF23 and FGFR. Now we propose that the glucuronized-steroid would be a lead compound of chemical drugs for patients with FGF23- and α -Kl-dependent rickets.

- (1) Imura A et al., Science 2007; 1615:1618
(2) Tomiyama K et al., PNAS 2010; 1666:1671

Nothing to Disclose: RM, AI, Y-IN

Pub # P1-214

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Genetic Ablation of Sfrp4 in Mice Does Not Affect Serum Phosphate Homeostasis

Author String M Christov, S Koren, Q Yuan, R Baron, B Lanske
Harvard School of Dental Medicine, Boston, MA; Beth Israel Deaconess Medical Center, Boston, MA;
Harvard School of Dental Medicine, Boston, MA

Body Serum phosphate levels are regulated by PTH and the FGF23/Klotho endocrine system, which both affect expression of Npt2a, and thus the apical re-absorption of phosphate in the proximal renal tubules. In addition to FGF23, secreted frizzled-related protein 4 (sFRP4) has recently been implicated as an additional phosphate regulator in vivo and in vitro. Here we demonstrate that ablation of the Sfrp4 gene in mice does not lead to altered serum or urine phosphate levels. Furthermore, Sfrp4 is unable to compensate for the absence of Fgf23 or Klotho, as double knockouts have a similar biochemical profile and phenotype as animals with ablation of Fgf23 or Klotho alone. Taken together, our data suggest that Sfrp4 does not contribute to the long-term regulation of serum phosphate levels in mice.

Sources of Research Support: MC is supported by NIDDK T32 training grant DK007199-33; BL is supported by NIDDK grant DK073944.

Nothing to Disclose: MC, SK, QY, RB, BL

Pub # P1-215
Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)
Title Osteopromoting Effects of rhIGF-I in a Mouse Model of Type 1 Diabetes
Author String J Fowlkes, J Nyman, C Jo, E Wahl, L Liu, G Cockrell, L Clark, C Lumpkin, K Thraillkill
University of Arkansas for Medical Sciences, Little Rock, AR; Vanderbilt University Medical Center,
Nashville, TN; Scott & White Healthcare, Temple, TX; Arkansas Children's Hospital Research Institute, Little
Rock, AR

Body Type 1 diabetes (T1D) is associated with an increased risk for fracture and poor bone healing during fracture repair. Using a streptozotocin (STZ)-induced mouse model of T1D we have demonstrated that diabetes produces: 1) inhibition of regenerative bone formation during tibial distraction osteogenesis (DO); and 2) progressive perturbations in skeletal integrity, including decreases in cortical thickness, bone mineral density and bone toughness. These abnormalities have been attributed to the hyperglycemic environment present in untreated diabetes, which can be partially rectified with insulin replacement. However, T1D is associated not only with a deficiency of pancreatic insulin secretion but with a secondary deficiency of IGF-I due to intraportal insulinopenia. Therefore, we examined the effects of systemic IGF-I treatment on bone formation and bone quality in diabetic mice. STZ or vehicle (10 mM citrate buffer) was injected into female CD-1 mice at 10 weeks of age (40 mg/kg/day x 5 days) to induce diabetes. Twenty-one days after STZ, diabetic (T1D) or control (CON) mice were then treated with rhIGF-I (1.5 mg/kg/day as subcutaneous infusion via Alzet minipump) or vehicle (PBS) throughout a 14 day DO procedure. At sacrifice, trunk blood was assayed for glucose, insulin, rhIGF-I, leptin and resistin. Distracted tibiae were analyzed radiographically to assess bone formation. Contralateral femurs were assessed by [micro]CT and biomechanical testing. Glucose concentrations at sacrifice were 100±5 (CON), 105±7 (CON+IGF), 495±39 (T1D) and 268±36 (T1D+IGF) mg/dl. New bone in the DO gap was reduced radiographically in T1D mice (CON: 68±4 vs. T1D: 25±10%; $p=0.001$) but improved with IGF-I treatment (T1D+IGF: 47±7%, $p=0.04$). Evaluation of the contralateral femurs demonstrated significant reductions in trabecular thickness, yield strength and peak force, which were also improved with IGF-I treatment. IGF-I also reduced bone porosity in both CON and T1D mice. Treatment with rhIGF-I did not correct concurrent deficiencies of the osteo-promoting hormone, insulin, or the adipokines, leptin and resistin. These findings demonstrate that despite persistent hyperglycemia, insulinopenia and adipokine deficiencies, rhIGF-I promotes new bone formation and improves biomechanical properties of bone in a model of T1D, suggesting it may be useful as a fracture preventative in this disease.

Sources of Research Support: Children's University Medical Group fund of the Arkansas Children's Hospital Research Institute (to KMT); Martha Ann Pugh Diabetes Research Fund (to KMT); Arkansas Biosciences Institute (to JLF); Sturgis Charitable Trust for Diabetes Research (JLF); National Institutes of Health Grants R01DK055653 (to JLF), R01AA012223 (to CKL), and C06RR16517 (to Arkansas Children's Hospital Research Institute).

Nothing to Disclose: JF, JN, CJ, EW, LL, GC, LC, CL, KT

Pub # P1-216

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title GH and IGF-I Supplementation Increase Cortical Bone Traits in ALS-Deficient Mice

Author String HW Courtland, H Sun, M Bet-On, S Yakar
Mount Sinai School of Medicine, New York, NY

Body Previously we found that the acid labile subunit (ALS) knockout (ALSKO) mice with reduced serum IGF-1 levels (~50% lower than control levels by 16 weeks) show significantly reduced body weight and bone size. To determine if reductions in body and skeletal size can be counteracted by hormone replacement, we treated 8 week old male ALSKO mice with GH (1 mg/kg), IGF-1 (1 mg/kg), or both GH and IGF-1 (double) for 8 weeks. We found that after 8 weeks of treatment, body weight, body composition (lean/fat mass) and femoral length, did not differ between the hormone and vehicle treated groups, despite a ~20% increase in serum IGF-1 levels in all treated groups.

Micro-CT analysis revealed that GH or double hormone (GH+IGF-1) treatments increased femoral total area (Tt.Ar, ~14%) and cortical area (Ct.Ar ~9%). Cortical thickness (Ct.Th ~4.5%) and marrow area (Ma.Ar ~17%) were also significantly increased, but only in double-treated and GH-treated animals, respectively. Consequently, GH and double-treated bones were more robust (less slender) than femora from vehicle treated mice. Trabecular bone volume per total volume (BV/TV) from the femoral distal metaphysis was identical for all groups as was bone mineral density (BMD).

The results of this study indicate that GH treatment can increase cortical bone size and amount in ALS-deficient mice without changes in body weight or body length. Double treatment (GH+IGF-1), although found to be effective, did not differ from GH treatment alone, while IGF-1 treatment alone did not enhance body size or skeletal size.

Given that serum IGF-1 levels were identically elevated in all treatment groups, we conclude that GH affects skeletal size directly via the GHR in bone tissue or indirectly via modulation of tissue IGF-1 levels.

Sources of Research Support: NIH Grants AR054919 & AR055141 to SY.

Nothing to Disclose: HWC, HS, MB-O, SY

Pub # P1-217

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Loss of ALS Protects Against Cortical Bone Loss in the Aging Mouse Skeleton

Author String HW Courtland, H Sun, S Elis, S Yakar
Mount Sinai School of Medicine, New York, NY

Body In the absence of the acid labile subunit (ALS), mouse serum IGF-1 levels are markedly reduced, resulting in mild growth retardation as well as significant reductions in bone mass and bone size by adulthood. However, to date, the role of ALS and serum IGF-1 in age-related bone loss remains unclear. To determine if an absence of ALS, and thus reductions in serum IGF-1, affect bone loss during aging we studied acid-labile subunit knockout (ALSKO) and control (C57BL/6J) male mice at 52, 80 and 104 weeks of age. At all ages control mice were significantly heavier (~16-18%) and had significantly greater serum IGF-1 levels (~51-54%) than ALSKO mice. However, at 52 and 80 weeks ALSKO mice indicated no differences in femoral total area (Tt.Ar), cortical area (Ct.Ar), or marrow area (Ma.Ar), indicating that male ALSKO mice can [ldquo]catch up [rdquo] to control mice between 16 and 52 weeks of age and maintain these skeletal traits into early aging. During later aging (104 weeks) we found that control mice had significantly increased Tt.Ar (2.85+/-0.37mm² vs 2.26+/-0.18mm² in ALSKO mice), Ma.Ar (1.79+/-0.29mm² vs 1.40+/-0.24mm² in ALSKO mice), and Ct.Ar (1.06+/-0.12mm² vs 0.86+/-0.10mm² in ALSKO mice) as compared to ALSKO mice. Concurrent with these changes was a significant increase in intracortical porosity in control mice as compared to ALSKO mice (3.02+/-2.77% vs 0.16+/-0.42% in ALSKO mice). Thus, increases in cortical bone size and amount (predominantly from periosteal apposition) appear to be a compensatory mechanism that offsets the reduction in mechanical stability that would otherwise result from a significant loss of intracortical bone. Given that ALSKO mice did not show significant increases in intracortical porosity from 52 to 104 weeks of age, a loss of ALS appears to protect against age-related bone loss directly or indirectly through changes in serum IGF-1 levels. Interestingly, at 104 weeks, where ALSKO mice are protected from intracortical bone loss, GH levels are significantly higher than in control mice, suggesting that regulation of GH (i.e., increases being protective may be crucial in this regulation of age-related cortical bone loss.

Sources of Research Support: NIH Grants AR054919 & AR055141 to SY.

Nothing to Disclose: HWC, HS, SE, SY

Pub # P1-218

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title *In Vivo* and *Ex Vivo* Effects of Metformin on Alterations of Bone Metabolism Associated with Fructose-Induced Metabolic Syndrome in Rats

Author String JI Felice, C Sedlinsky, AM Cortizo, MS Molinuevo, MV Gangoiti, MJ Tolosa, L Schurman, AD McCarthy
Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina

Body Several clinical studies have shown that the Metabolic Syndrome (MS) is associated with a decrease in bone mass and with an increase in the rate of osteoporotic fractures. We have recently found that orally administered metformin induces osteogenic effects in rats, promoting the osteoblastic commitment of bone marrow progenitor cells (BMPC) and increasing bone lesion repair.

In the present study we evaluated the effects of fructose-induced MS on bone metabolism in rats, and the possible modulation of these effects by orally administered metformin. Male young Sprague Dawley rats (200g) were divided into 4 groups: C (no treatment), M (100mg/kg body weight/day Metformin in drinking water), F (10% Fructose in drinking water) and FM (Fructose+Metformin in drinking water). All treatments were continued for 3 weeks, after which blood samples were taken prior to animal sacrifice by neck dislocation under anesthesia. Femurs were immediately dissected to obtain BMPC and for evaluation of metaphyseal microarchitecture (trabecular volume and osteocytic density) by hematoxylin-eosin staining. BMPC were cultured in an osteogenic medium (with ascorbic acid and beta-glycerophosphate) for 2-3 weeks and then evaluated for alkaline phosphatase activity (ALP), type 1 collagen secretion and extracellular mineralization.

An increase in non-fasting blood glucose and triglycerides was observed in F versus C group, compatible with the onset of MS. After osteogenic induction, BMPC from F group showed a significant decrease in ALP (82% of C, $p<0.05$), type 1 collagen secretion (62% of C, $p<0.01$) and mineralization (81% of C, $p<0.05$). Metformin co-treatment (FM group) did not modulate this fructose-induced decrease in ALP; however, it partially prevented the decrease in collagen secretion (74% of C) and completely abrogated the fructose-induced inhibition of mineralization (103% of C). Analysis of femoral metaphyses showed that fructose alone induced a decrease in trabecular volume (88% of C, $p<0.05$) and in osteocyte density (79% of C, $p<0.05$). Metformin co-treatment (FM group) showed a tendency to prevent these decreases (96% of C for trabecular volume; 88% of C for osteocyte density).

These results show for the first time, (a) that fructose-induced MS in rats decreases the osteogenic potential of BMPC, additionally impairing trabecular bone microarchitecture; and (b) that these deleterious effects on bone can be partially or totally prevented by oral metformin treatment.

Nothing to Disclose: JIF, CS, AMC, MSM, MVG, MJT, LS, ADM

Pub # P1-219

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Skeletal Muscle Contraction Using Functional Electrical Stimulation Reduces Effects of Unloading on Bone Resorption Independently from the Central Nervous System: Studies in a Rat Model of Complete Spinal Cord Transection

Author String W Qin, L Collier, Y Peng, Y Wu, L Sun, G Creasy, J Li, Y Qin, WA Bauman, J Jarvis, M Zaidi, C Cardozo James J Peters VA Medical Center, New York, NY; Mount Sinai School of Medicine, New York, NY; Mount Sinai School of Medicine, New York, NY; Palo Alto Health Care System, Palo Alto, NY; Mount Sinai School of Medicine, New York, NY; University of Liverpool, Liverpool, UK

Body Complete spinal cord injury (SCI) causes severe bone loss and disrupts connections between higher centers in the central nervous system (CNS) and bone. Muscle contraction elicited by functional electrical stimulation (FES) partially protects against loss of bone but the cellular and molecular events by which this phenomenon occurs are unknown. Using a rat model, the effects of 7 days of contraction-induced loading of tibia and fibula by FES, begun 16 weeks after SCI, were characterized. SCI reduced tibial and femoral BMD by 12-17% and promoted bone resorption, as indicated by increased serum C-terminal cross-linking telopeptide of type I collagen (CTX); SCI-related changes in CTX were reversed by FES. In cultures of bone marrow cell-derived cells, SCI increased the number of osteoclasts and mRNA levels of the osteoclast differentiation markers calcitonin receptor, integrin beta 3 and TRAP; these changes were significantly reduced by FES. The number of osteoblasts arising from cultured bone marrow cells was also reduced by SCI as was the ratio of OPG/RANKL mRNAs in these cells; the unfavorable reduction in the OPG/RANKL ratio was partially reversed by FES. In osteoblasts, SCI increased mRNA levels of the Wnt inhibitors DKK1, sFRP2 and SOST. FES completely reversed the SCI-induced change for DKK1 and sFRP2, and partially reversed it for SOST. Our results demonstrate an anti-bone resorptive effect of acute FES after SCI and suggest a mechanism involving increased Wnt signaling, likely a result of reduced expression of Wnt inhibitors, to cause more favorable ratios of OPG/RANKL and the inhibition of osteoclastogenesis. The present study indicates that the effects of bone reloading on SCI-related bone remodeling occurred independently of the effects of higher CNS centers on bone.

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Nothing to Disclose: WQ, LC, YP, YW, LS, GC, JL, YQ, WAB, JJ, MZ, CC

Pub # P1-220

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Central Deletion of Brain-Derived Neurotrophic Factor Induces Obesity and High Bone Mass

Author String C Camerino, P Hauschka, M Zayzafoon, M Rios
Harvard University, Boston, MA; Children's Hospital, Boston, MA; University of Alabama, Birmingham, AL
Tufts University, Boston, MA

Body Brain Derived Neurotrophic Factor (BDNF), is essential for neuronal survival and plasticity. It is also involved in the regulation of food intake, the pathobiology of obesity, and type 2 diabetes mellitus. BDNF acts in an insulin-like fashion lowering blood glucose to normoglycemic levels in obese diabetic models when administered systemically. Recent studies indicated that factors that alter blood sugar also influence bone resorption. To determine the effect of BDNF on bone mass and metabolism we evaluated BDNF conditional knock out mice, in which BDNF expression was deleted from the brain through the use of the Cre-loxP recombination system under the direction of the α -calcium/calmodulin-dependent protein kinase 2 promoter (Rios et al, 2001). In both males and females, at 3 and 6 mo of age, central BDNF deletion leads to a metabolic phenotype characterized by hyperphagia, increased body weight, elevated serum levels of insulin and glucose, and leptin resistance. The mice are hyperactive with unchanged expression level of UCP1 in brown adipose tissue. Regarding the skeletal phenotype, BDNF KO mice have increased femur length (+6%, $p < 0.01$) and greater femoral bone mineral density (BMD +30%, $p < 0.001$) and content (BMC +50%, $p < 0.001$) [micro]CT analysis of the femurs and vertebrae of 3 mo old BDNF KO mice reveal significantly greater trabecular bone volume (BV/TV +50% for distal femur, $p < 0.001$ +35% for vertebral body, $p < 0.001$) and mid-femoral cortical thickness (+11 to 17%, $p < 0.05$). Further studies will be aimed to understand if BDNF may be of therapeutical merit to design a single therapy that concurrently target osteoporosis and type-2 diabetes.

Rios et al., Mol Endocrinol 2001; 15:1748

Nothing to Disclose: CC, PH, MZ, MR

Pub # P1-221

Session Information POSTER SESSION: BASIC - Bone, Calcitropic Hormones & Vitamin D (1:30 PM-3:30 PM)

Title Calcium Transporters, PMCA1, NCKX3, and Calbindin-28k, Are Distinctly Expressed in Human Placental BeWo Cells

Author String H Yang, E-B Jeung
College of Veterinary Medicine, Chungbuk National University, Cheongju, Korea

Body During last trimester of gestation, calcium (Ca^{2+}) transport from mother to fetus increases dramatically in response to the accelerated demand for Ca^{2+} caused by bone mineralization in the fetus. Regarding the molecular basis of Ca^{2+} transport, several studies suggest that transcellular active Ca^{2+} transport is comprised of three processes: (1) the apical Ca^{2+} entry, (2) the binding with calbindins that serves as an intracellular Ca^{2+} buffer, and (3) basolateral exit. In this study, cell membrane and cytosolic calcium transporters, i.e., TRPV6, PMCA1, NCKX3 and Calbindin-D28k (CaBP-28k), were investigated at induced oxidative stress in human placental BeWo cells. In hypoxia, human placental expressions of TRPV6 mRNA and protein were not altered in BeWo cells, however, other calcium transporters including NCKX3, CaBP-28k were increased at hypoxic condition in BeWo cells compared to a control (normoxia). In addition, the expression of PMCA1 mRNA and protein was suppressed at hypoxia BeWo cells. Taken together, these results showed that calcium transporters, i.e., TRPV6, PMCA1, NCKX3 and CaBP-28k, are distinctly regulated by induced oxidative stress in BeWo cells, suggesting that alterations of calcium transporters in hypoxic stress may be involved in placental function by regulating calcium transfer in human placenta.

Nothing to Disclose: HY, E-BJ

Pub # P1-222

Session Information POSTER SESSION: CLINICAL - Calcium Metabolism, Therapeutics (1:30 PM-3:30 PM)

Title The Role of Insulin on Urine Calcium Excretion

Author String V Yoon, NM Maalouf, OW Moe, K Sakhaee
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Body **Background:** Epidemiologic studies have shown an increased risk of kidney stone formation with obesity and weight gain. Several short-term metabolic studies have shown a postprandial rise in urine calcium excretion. However, it is not known if insulin or glucose mediates this postprandial hypercalciuria. We sought to examine the effects of hyperinsulinemia independent of glucose on urinary calcium excretion during a 2 hour hyperinsulinemic euglycemic clamp.

Methods: Fifteen non-stone formers were recruited across a range of body mass index (7 lean, 8 obese). Fasting serum and 2hr urine were collected prior to and during the clamp procedure.

Results: There was a significant rise in serum insulin levels from prior to during the clamp (8 ± 3 vs. 131 ± 28 mIU/mL, $p < 0.001$), and there was no change in serum glucose (91 ± 7 vs. 92 ± 8 mg/dL, $p = 0.51$). Despite the lack of change in serum calcium concentration (8.8 ± 0.3 vs. 8.8 ± 0.3 mg/dL, $p = 0.1$), there was a significant rise in fractional excretion of calcium (FeCa) from baseline to hyperinsulinemia (1.0 ± 0.6 vs. $1.3 \pm 0.6\%$, $p = 0.0006$). There was no difference between lean and obese subjects in the rise in FeCa (0.3 ± 0.2 vs. $0.3 \pm 0.3\%$, $p = 0.95$). FeCa did not correlate with insulin sensitivity, as measured by glucose disposal rate ($r^2 = 0.0169$, $p = 0.64$). There was a small but significant rise in FeNa in all subjects (0.04 ± 0.02 vs. $0.06 \pm 0.02\%$, $p = 0.009$), however FeCa rise was independent of the rise in urinary sodium excretion.

Conclusions: Hyperinsulinemia increases urine calcium excretion independent of changes in serum glucose. For the first time, we showed the rise in urine calcium in response to insulin was also independent of body weight, degree of insulin sensitivity, and urine sodium excretion. Hyperinsulinemia may potentiate calcium stone risk through hypercalciuria regardless of body weight.

Nothing to Disclose: VY, NMM, OWM, KS

Pub # P1-224

Session Information POSTER SESSION: CLINICAL - Calcium Metabolism, Therapeutics (1:30 PM-3:30 PM)

Title An Unusual Cause of Malignancy-Related Hypercalcemia

Author String M-A Doyle, J Malcolm
University of Ottawa, Ottawa, Canada

Body **Background:** Hypercalcemia is a well established paraneoplastic condition that is most commonly due to plasma factor PTH-rP. Few cases of PTH-secreting tumours have been reported in the literature. We present a rare case of malignancy associated hypercalcemia secondary to PTH.

Clinical Case: A previously healthy 28-year-old woman was admitted to hospital with hypercalcemia (Ca = 4.11 mmol/L N = 2.20-2.52 mmol/L) and acute renal failure (Creatinine=215 umol/L N= 35-88 umol/L) after a 2-week history of nausea, fatigue, abdominal pain and weight loss. Following therapy with IV fluids, Pamidronate and Calcitonin, her calcium had improved to 2.82 mmol/L. PTH was found to be elevated at 24.6 pmol/L (N = 1.6-9.3 pmol/L) raising the possible diagnoses of primary hyperparathyroidism or MEN1. An abdominal U/S revealed a 60 x 67 x 80mm solid mass between the stomach and pancreas and multiple liver metastases.

Biopsy of the liver metastases revealed a poorly differentiated neuroendocrine carcinoma small-cell type. Further staging investigations including pituitary MRI and genetic testing for MEN1 were negative and a CT-scan of the neck and sestamibi scan were negative for a parathyroid adenoma. Treatment with Cis-platinum/Etoposide resulted in a decrease in the pancreatic mass (56 x 49mm) and liver metastases. Calcium levels normalized with shrinkage of the tumour.

A few months later she was readmitted with a calcium of 3.23 mmol/L. A CT-scan of the abdomen showed an increase in the pancreatic mass (67 x 58mm). Her PTH level increased to 48.2 pmol/L. PTH-rP was within normal limits (7.8 pg/ml N = 1-15 pg/ml). The patient was given a trial of Cinacalcet and after the third dose her calcium decreased to 2.63 mmol/L. However she did not tolerate it and it was discontinued. Chemotherapy was restarted and after the first cycle her calcium levels had normalized (Ca 2.34 mmol/L) and after the second cycle PTH levels had decreased (16.6 pmol/L). A repeat CT scan of her abdomen showed a decrease in the pancreatic mass (49 x 42mm).

Clinical Lessons: PTH secreting tumours, although rare, should be considered when PTH levels are elevated and there is no parathyroid related cause found. Hypercalcemia due to PTH-secreting tumours may be more resistant to standard treatment. Calcimimetics may be of benefit in controlling calcium levels in these patients. Recognizing that elevated PTH levels may be associated with malignancy may lead to earlier detection of an undiagnosed malignancy.

Nothing to Disclose: M-AD, JM

Pub # P1-225

Session Information POSTER SESSION: CLINICAL - Calcium Metabolism, Therapeutics (1:30 PM-3:30 PM)

Title [ldquo]A Perfect Storm for Hypocalcemia[rdquo]: Malabsorption, ESRD and Hypoparathyroidism

Author String BM Palal, S Reutrakul, M Sinsakul
Rush University Medical Center, Chicago, IL; Rush University Medical Center, Chicago, IL

Body Bariatric surgery employing Roux-en Y gastric bypass (RYGB) results in the bypass of duodenum and jejunum, preferential sites for vitamin D and calcium absorption. The risks of vitamin D deficiency, hypocalcemia and subsequent secondary hyperparathyroidism are increased following RYGB. Therefore, these patients, when undergoing procedures that affect the parathyroid glands, are at even higher risk of severe symptomatic hypocalcemia.

Case Report: A 54 year-old woman underwent RYGB 5 years ago due to morbid obesity. She developed ESRD requiring hemodialysis and subsequently calciophylaxis. Serum calcium was 9.8 mg/dl, and intact PTH was 2466 pg/ml. She underwent 3-[frac12] gland parathyroidectomy. Post-op serum calcium was 7.0 mg/dl and she was discharged on calcitriol 0.25 mcg BID and calcium carbonate 3000 mg TID.

Seven weeks later she presented with generalized weakness, perioral numbness and leg cramps. She had a positive Chvostek sign and a prolonged QT-interval. Serum calcium level was 5.4 mg/dl with PTH 87 pg/ml and 25-OH vitamin D level was 16 ng/ml. She was stabilized with an IV calcium infusion, and continued on oral calcitriol and calcium carbonate. She had recurrent hypocalcemia upon weaning of IV calcium, and therefore calcitriol was gradually increased to 3 mcg BID, and calcium carbonate was changed to a suspension at 2000 mg QID. Calcium citrate suspension 400mg tid and ergocalciferol, 50,000 units weekly, was also added. She required IV calcium for 18 days before serum calcium level stabilized on the oral regimen. On the same regimen 9 months later serum calcium remained normal at 9.7 mg/dl.

Conclusion: Our patient developed severe prolonged hypocalcemia and required very large doses of oral calcium carbonate and calcitriol to stabilize serum calcium levels. This was likely due to a combination of malabsorption, ESRD, hypoparathyroidism and hungry bone syndrome. Clinicians should be aware that if RYGB patients subsequently undergo neck surgery and develop post-operative hypoparathyroidism, they may develop severe hypocalcemia and may require much larger than usual doses of calcium and calcitriol supplementation for stabilization.

Nothing to Disclose: BMP, SR, MS

Pub # P1-226

Session Information POSTER SESSION: CLINICAL - Calcium Metabolism, Therapeutics (1:30 PM-3:30 PM)

Title Serum Phosphate Predicts Temporary Hypocalcemia after Thyroidectomy

Author String AH Sam, WS Dhillon, M Donaldson, A Moolla, K Meeran, NS Tolley, F Palazzo
Hammersmith Hospital, Imperial College London, London, UK; Imperial College Healthcare NHS Trust, London, UK; St Mary's Hospital, Imperial College Hospitals NHS Trust, London, UK; Hammersmith Hospital, Imperial College Hospitals NHS Trust, London, UK

Body

Background: Temporary hypocalcaemia occurs in up to 40% of patients following a total thyroidectomy. Serum calcium and parathyroid hormone (PTH) measurements are currently used to predict post-thyroidectomy hypocalcaemia. However, immediate access to PTH measurement is expensive and not widely available. Serum phosphate responds rapidly to changes in circulating PTH levels and its measurement is readily available in all hospitals. We evaluated the use of serum phosphate to predict temporary hypocalcaemia post-thyroidectomy.

Methods: We retrospectively assessed 111 consecutive patients who had total or completion thyroidectomy. Patients had serum calcium and phosphate measured pre-operatively, on the evening of surgery (day 0), on the morning of day 1 and over the following week as clinically indicated. Serum PTH was measured on the morning of day 1. Vitamin D levels were measured pre-operatively.

Results: Seventy-six patients did not develop treatment-demanding hypocalcaemia. In these patients, the mean serum phosphate concentration was lower on the morning of day 1 compared to that on the evening of surgery. Seventeen patients with a vitamin D > 25 nmol/l developed hypocalcaemia requiring treatment from day 1 onwards. All had an overnight rise in serum phosphate to > 1.44 mmol/l (100% sensitivity and specificity for predicting hypocalcaemia). Twelve patients who had a vitamin D < 25 nmol/l also developed hypocalcaemia but had an attenuated rise in serum phosphate.

Conclusion: Serum phosphate is a reliable biochemical predictor of post-thyroidectomy hypocalcaemia in patients without vitamin D deficiency. Use of serum phosphate may facilitate safe day 1 discharge of patients undergoing thyroidectomy.

Nothing to Disclose: AHS, WSD, MD, AM, KM, NST, FP

Pub # P1-227

Session Information POSTER SESSION: CLINICAL - Calcium Metabolism, Therapeutics (1:30 PM-3:30 PM)

Title Biochemical Predictors of Postparathyroidectomy Hypocalcemia in Chronic Kidney Disease (Rajavithi Hospital Results)

Author String S Niramitmahapanya, T Sunthornthepvarakul, C Deerochanawong, V Sarinnapakorn, P Athipan
Rajavithi Hospital, Medical College of Rangsit University, Bangkok, Thailand; Rajavithi Hospital, Medical College of Rangsit University, Bangkok, Thailand

Body

Objective: To predicted clinical and biochemical factors in hypocalcemia of chronic kidney disease with hyperparathyroidism (CKD-HPT) patients who were referred to parathyroidectomy.

Material and Method: Retrospective study was performed in 54 adults CKD patients who underwent parathyroidectomy in Rajavithi Hospital during September 2001 to August 2010. Serum calcium was drawn a pre-operative day and followed drawn daily by seven days postoperative period. Post-operative hypocalcemia was defined by post-operative mean serum-corrected calcium level was below 8 mg/dl and clinical sign of hypocalcemia was indicated that needed intravenous calcium gluconate infusion.

Results: Records of 54 hyperparathyroidism patients who underwent parathyroidectomy by chronic kidney disease were reviewed. We found that 44 patients (81.5%) had hypocalcemia in post-operative parathyroidectomy. In CKD-HPT patients age, serum phosphate level, calcium-phosphorus product and decrement of serum iPTH level were significant predictors of hypocalcemia with statistic significant ($p=0.017$, 0.015 , 0.021 and 0.032 respectively) in HPT patients who need intravenous calcium-gluconate infusion. In multiple linear-logistic regression analysis of CKD-HPT; phosphate level and decrement of serum iPTH level are independent predictors of hypocalcemia postparathyroidectomy with statistical significance at $p=0.024$ and <0.005 respectively.

Conclusion: Hypocalcaemia postparathyroidectomy was common problem in chronic kidney disease. Preoperative predictors such as serum phosphate and iPTH level should be monitored by physicians to preven hypocalcaemia event in parathyroidectomy patients.

Nothing to Disclose: SN, TS, CD, VS, PA

Pub # P1-228

Session Information POSTER SESSION: CLINICAL - Calcium Metabolism, Therapeutics (1:30 PM-3:30 PM)

Title Vitamin D Insufficiency Is Associated with Decreased Bisphosphonate Response

Author String A Shieh, A Carmel, RS Bockman
New York-Presbyterian Hospital/Weill Cornell, New York, NY; Hospital for Special Surgery, New York, NY

Body

Background: Randomized clinical trials have shown bisphosphonates to increase bone mineral density (BMD) and prevent fractures. It is unclear, however, why patients outside of these settings do not respond to bisphosphonates at comparable rates. The aim of this study was to explore the association between vitamin D status and response to bisphosphonates.

Methods: A retrospective chart review of patients from a specialty osteoporosis practice was completed to identify subjects who were female; post-menopausal; had been taking alendronate, risedronate, ibandronate or zoledronate for ≥ 18 months; and had undergone ≥ 2 DXA scans separated by 18 to 60 months. Patients were excluded for chronic steroid use; metabolic bone disease; chronic kidney disease; or non-adherence to bisphosphonates. Data collected were age; BMI; bisphosphonate taken/treatment duration; concurrent calcium supplementation; fracture prior to/during bisphosphonate therapy; BMD and T-score at the lumbar spine/femoral neck/trochanter/total hip from the two most recent DXA scans; bone markers and 25-hydroxyvitamin D (25OHD) measurements obtained with/between the two most recent DXA scans. Patients were categorized as responders or non-responders (defined as 1) $>3.0\%$ decrease in BMD at any site between initial/follow-up DXA scans; 2) incident low-trauma fracture despite >12 months of bisphosphonate use; or 3) T-score <-3.0 at any site despite ≥ 24 months of bisphosphonate use). Rates of vitamin D insufficiency (mean 25OHD <33 ng/mL) were compared between responders and non-responders. Patients were also stratified into quartiles by 25OHD level and rates of non-response were determined for each quartile.

Results: 160 patients met criteria, of whom 89 were responders, and 71 were non-responders (decreased BMD: 42; incident fracture: 17; persistently low T-score: 12). 16.8% of responders were vitamin D insufficient compared to 54.9% of non-responders ($p<0.0001$). Patients with 25OHD concentrations of <20 ng/mL, 20-30 ng/mL, 30-40 ng/mL, and >40 ng/mL had non-response rates of 83.3% (10/12), 77.8% (21/27), 42.3% (22/52) and 24.6% (17/69) respectively. 25OHD levels ≥ 33 ng/mL was associated with an increased odds of response (OR=7.24, 95% CI 3.44-14.82, $p<0.0001$).

Conclusions: Serum 25OHD >33 ng/mL was associated with a significant >7 -fold increased odds of adequate response to bisphosphonates. Vitamin D status should be optimized to achieve maximal benefit from bisphosphonate therapy.

Nothing to Disclose: AS, AC, RSB

Pub # P1-229

Session Information POSTER SESSION: CLINICAL - Calcium Metabolism, Therapeutics (1:30 PM-3:30 PM)

Title ACE-031 (Soluble Activin Receptor Type IIB-IgG1) Increases Muscle Mass by Inhibiting Myostatin and Other Negative Regulators of Muscle: Non-Clinical and Clinical Studies

Author String KM Attie, JL Lachey, Y Yang, D Wilson, E Haltom, ML Sherman
Accelaron Pharma, Cambridge, MA; Accelaron Pharma, Cambridge, MA

Body
Introduction. Myostatin is a potent inhibitor of muscle development, expressed post-natally exclusively in skeletal muscle. Congenital loss of myostatin leads to marked increases in muscle mass. Additional members of the TGF- β superfamily also act as negative regulators of muscle through the activin receptor type IIB (ActRIIB). ACE-031 is an investigational ActRIIB-IgG fusion protein therapeutic designed to act as a decoy receptor to bind myostatin and related ligands and block their signaling through endogenous ActRIIB receptors. ACE-031 is also designed to bind ligands that are involved in the regulation of other tissues, including fat and bone. ACE-031 is currently being developed to treat the neuromuscular disease Duchenne muscular dystrophy (DMD).
Non-clinical Studies. Animal studies have shown increased muscle mass, strength, and function in various models of neuromuscular disease, including the *mdx* mouse model for DMD. Studies using the murine version of ACE-031 (RAP-031) in *mdx* mice and/or other animal models have demonstrated increased lean mass (skeletal muscle), increased grip strength, decreased fibrosis and fat in muscle, increased utrophin expression, decreased creatine kinase, increased bone mass, and decreased fat mass.
Clinical Studies. Two phase 1, double-blind, placebo-controlled, studies (single ascending dose and multiple ascending dose, MAD) have been completed in healthy post-menopausal women (total n=118). In these studies, ACE-031 was generally well tolerated. Most adverse events were mild and transient, and included injection site erythema, headache, and epistaxis. Studies were not powered for pharmacodynamic (PD) endpoints. Preliminary PD data in the MAD study included the following mean maximum % changes from baseline: total body lean mass (DXA) increased 5.2% and mean thigh muscle volume (MRI) increased 4.1% in the 1 mg/kg q2wk group at Day 36. Total body fat mass decreased 8.2% in the 3 mg/kg q4wk group at Day 113. Bone biomarkers CTX decreased 55.4% in the 2 and 3 mg/kg q4wk groups at Day 57 and BSAP increased by 26.3% in the 1 mg/kg q4wk group at Day 15.
Conclusions. Preliminary data demonstrate that ACE-031 was generally well-tolerated in humans and had salutary effects on muscle, fat and bone. These results support further studies of ACE-031 in neuromuscular diseases to investigate its potential to improve muscle mass, strength, and function. A phase 2 study of ACE-031 in DMD is ongoing.

Sources of Research Support: Accelaron Pharma Inc.

Disclosures: KMA: Employee, Accelaron Pharma. JLL: Employee, Accelaron Pharma. YY: Employee, Accelaron Pharma. DW: Employee, Accelaron Pharma. EH: Employee, Accelaron Pharma. MLS: Employee, Accelaron Pharma.

Pub # P1-230

Session Information POSTER SESSION: CLINICAL - Calcium Metabolism, Therapeutics (1:30 PM-3:30 PM)

Title Bone Density Response to Teriparatide May Depend on Prior Bisphosphonate Use and Bisphosphonate-Free Interval

Author String P Dahl, A Shieh, RS Bockman
New York-Presbyterian Hospital/Weill Cornell, New York, NY; Hospital for Special Surgery, New York, NY

Body **Background:** Teriparatide has been shown to increase vertebral and femoral bone mineral density and reduce fracture risk. However, the anabolic effects of teriparatide can be attenuated by prior or concomitant bisphosphonate (BSP) therapy. Further, the optimal [ldquo]washout[rdquo] period between bisphosphonate discontinuation and teriparatide initiation is unclear. The aim of this study was to compare the change in BMD after ≥ 21 months of teriparatide therapy between BSP-na[iuml]ve and BSP-free (≥ 1 year off BSP) patients versus recently treated (≤ 1 year off BSP) patients .

Methods: A retrospective chart review of patients in a specialty osteoporosis practice was completed to identify subjects with osteoporosis (by 2010 NOF criteria), who had received 21-24 months of teriparatide (20 [mu]g SQ daily). Patients were excluded for chronic steroid use; metabolic bone disease; and history of bilateral hip replacements. Full patient demographics at study inception; as well as BMD and T-score at the lumbar spine (LS)/femoral neck (FN)/total hip (TH) at initiation and completion of teriparatide therapy were recorded. Urinary N-telopeptide (UNTx), bone-specific alkaline phosphatase (BSAP), and 25-hydroxyvitamin D (25OHD) at ~4-month intervals from initiation to completion of teriparatide; as well as fracture incidence prior to/while on teriparatide were monitored. Change in BMD and rate of fracture were compared between BSP-na[iuml]ve and BSP-free patients versus recently treated patients.

Results: For this interim analysis, 49 patients were identified [16 BSP-na[iuml]ve, 10 BSP-free, 23 recently treated (mean BSP-free period=2.6 months)]. % change in LS BMD was $10.8 \pm 15.2\%$ (N=22) in BSP-na[iuml]ve and BSP-free subjects v. $6.4 \pm 7.1\%$ (N=21) in recently treated subjects (p=0.24). % change in FN BMD was $8.5 \pm 10.1\%$ (N=14) in BSP-na[iuml]ve and BSP-free subjects v. $2.0 \pm 5.0\%$ (N=14) in recently treated subjects (p=0.04). % change in TH BMD was $9.0 \pm 14.8\%$ (N=8) in BSP-na[iuml]ve and BSP-free subjects v. $5.4 \pm 9.6\%$ (N=14) in recently treated subjects (p=0.49). Neither % change in UNTx and BSAP (baseline to peak) nor time to reach peak values differed significantly between groups. Only one subject (BSP-na[iuml]ve) suffered a new fracture while on teriparatide.

Conclusions: A trend towards greater increase in LS and TH BMD, as well as a significantly greater increase in FN BMD were seen in BSP-na[iuml]ve and BSP-free subjects when compared to recently treated subjects. However, this interim analysis was limited by small sample size.

Nothing to Disclose: PD, AS, RSB

Pub # P1-231

Session Information POSTER SESSION: CLINICAL - Calcium Metabolism, Therapeutics (1:30 PM-3:30 PM)

Title Denosumab for Refractory Lymphoma-Associated Hypercalcemia

Author String E Makonnen, M Styner
University of North Carolina, Chapel Hill, NC; University of North Carolina, Chapel Hill, NC

Body Background: Lymphoma tumor cells can secrete excess 1,25-dihydroxyvitamin D, leading to severe hypercalcemia independent of the action of PTH or PTHrp¹. Frequently, multiple doses of IV bisphosphonate are required with a risk for adverse effects. Additionally, patients may become refractory to bisphosphonates after repeated administration. Thus, additional calcium lowering agents are desirable in this setting.

Case: A 64 year old Caucasian man with hypercalcemia due to lymphoma-associated excess 1,25(OH)₂D was treated with 60 mg IV pamidronate for a calcium of 12.1 mg/dl (ref: 8.5-10); GFR was 28.34 ml/min (nl >60 ml/min). His calcium declined to the 8.0 mg/dl range 10 days after bisphosphonate. One month later, he had recurrence of hypercalcemia to the 12 mg/dl range that failed to respond to IV Pamidronate. At this time chemotherapy was initiated for his B cell lymphoma including R-CHOP and etoposide. His labs were as follows: calcium 12.7 mg/dl, PTH < 4 pg/ml (12-72) , 1,25 OH vitamin D 160 pg/ml (nl 18-64), 25 OH Vitamin D 24 ng/ml (nl 25-80 ng/ml) and PTH-rp < 0.2 pmol/l (nl <2), albumin 2.2 g/dl (nl 3.5-5.0), GFR 21.5 ml/min.

To treat bisphosphonate refractory hypercalcemia in a patient with renal insufficiency we elected to administer denosumab 60 mg subcutaneously once, with disclosure to the patient regarding the off-label use of this agent. One week after receiving denosumab calcium was measured at 8.5 mg/dl. One month after denosumab, the patient suffered a seizure which we attributed to his hypocalcemia with calcium of 6.2 mg/dl. Of note, when the patient presented for his next cycle of chemotherapy he also developed right lower extremity cellulitis, a known complication of denosumab. The labs were as follows: calcium 6.7 mg/dl, albumin 3.1 g/dl, PTH 503 pg/ml, 1,25 OH vitamin D 17 ng/ml, 25-OH Vit D of 22 ng/ml, and GFR of 35.3 ml/min. After treatment with antibiotics and chemotherapy, he was discharged home on aggressive three time weekly IV calcium for 5 weeks and then switched to oral Calcium Carbonate 6,000 mg/day.

Conclusion: Denosumab rapidly and effectively lowered calcium in our patient with hypercalcemia due to elevated 1,25D due to lymphoma. This was followed with symptomatic and prolonged hypocalcemia as 1,25 D dropped successfully due to chemotherapy. The resultant secondary hyperparathyroidism failed to adequately raise the serum calcium, likely due to persistent effect of denosumab on bone resorption.

1-Andrew F. Stewart. Hypercalcemia associated with cancer. N Engl J Med 2005;352:373-9.

Nothing to Disclose: EM, MS

Pub # P1-232

Session Information POSTER SESSION: CLINICAL - Calcium Metabolism, Therapeutics (1:30 PM-3:30 PM)

Title Follow-Up of Atypical Fractures Associated with Long-Term Bisphosphonate Therapy: A Case Series

Author String FA Padilla, AV Joy, P Taxel, F Mirza, L Cirullo
University of Connecticut Health Center, Farmington, CT

Body

Objective: Describe a case series of 8 postmenopausal women with osteoporosis (OP) who sustained atypical fractures defined as transverse or oblique, subtrochanteric or femoral shaft (ST/FS) fractures, while on long-term bisphosphonate (BP) therapy.

Case series: Eight postmenopausal women experienced 10 atypical fractures of the femur while on long-term BP therapy. Their mean age was 71 years with a mean duration of BP therapy of 9 years. These patients had an average follow-up of 30 months post- fracture.

All patients received alendronate, and one was on ibandronate for 3 years preceded by alendronate for 7 years. T score was in osteopenic range in 4 patients, 2 were in the osteoporotic range and 2 were normal. Urine NTX available in 4 patients prior to the fracture was < 25 nmol BCE/mmol Cr. Of the 10 fractures, 6 were not preceded by trauma; 4 were related to falls. Pain along the thigh several days to months prior to the fracture was present in half of subjects. One patient had a right sided fracture after a fall and developed dull pain in the left thigh 2 months later; a bone scan showed a stress fracture in the mid shaft. Another developed bilateral thigh pain; an MRI showed focal cortical thickening in the lateral aspect of the proximal femoral diaphysis bilaterally, with marrow edema and periostitis compatible with incomplete stress fractures. This patient had a spontaneous transverse left ST fracture two weeks later. All fractures were treated surgically by internal rod fixation or intramedullary nailing, and all regained their prior functional status. Six patients stopped BPs immediately after the fracture while two continued for another two years. Two patients received teriparatide post-fracture; one received raloxifene and all continued calcium and vitamin D supplementation. No additional fractures have been reported in these patients at the current time.

Discussion: BP plays a central role in the management of OP as they decrease the risk of vertebral and hip fractures. In recent years, case reports have linked the use of alendronate to the development of ST/FS fractures. These fractures raise questions regarding the optimal duration of BP therapy, and management after sustaining these uncommon fractures. In patients with OP, we recommend a drug holiday after 5 years if the patient is at low risk for osteoporotic fractures. Long term studies are needed to determine if this clinical maneuver will decrease the frequency of these atypical fractures.

Nothing to Disclose: FAP, AVJ, PT, FM, LC

Pub # P1-233

Session Information POSTER SESSION: CLINICAL - Calcium Metabolism, Therapeutics (1:30 PM-3:30 PM)

Title Denosumab (Prolia) Induced Hypocalcemia in an 85-Year-Old Female

Author String ZM Mamish, SM Hammond, NL Knowles
Sacred Heart Health System, Pensacola, FL

Body

Background: Denosumab is a novel antiresorptive agent recently approved for the treatment of Postmenopausal Osteoporosis. It is a human monoclonal antibody that blocks RANK Ligand from binding to RANK on the surface of premature Osteoclasts, thereby inhibiting their maturation and activation. Here we report a case of Denosumab induced hypocalcaemia, which according to our knowledge has not been previously reported.

Clinical Case: An 85 year old Caucasian female with Osteoporosis presented with a DEXA showing a 13% decline in bone density. Past history is significant for Vitamin D deficiency, hyperthyroidism and Rheumatoid arthritis. Recently she underwent a gastrectomy for gastric cancer followed by radiation treatment. There is no previous history of renal impairment or hypocalcaemia. She was intolerant to oral Bisphosphonates and refused intravenous Zoledronic acid.

Initial labs showed a creatinine of 0.8 mg/dL (n:0.4-1.4 mg/dl), estimated GFR greater than 60 ml/minute, calcium 8.6 mg/dL (n:8.4-10.8 mg/dl), albumin 3.5 gm/dl (n:3.5-5.5 gm/dl), 25 hydroxy vitamin D 15 ng/ml (n:32-100 ng /ml). The patient reported adequate intake of calcium and Vitamin D. We added Vitamin D 50,000 units every 2 weeks. Three months later, we administered Denosumab 60 mg injection. One week later she presented with complaints of paresthesias, cramps and numbness of the lips. Labs showed calcium of 5.9 mg/dL. She was given Paricalcitol 2 mcg orally twice daily for 12 days and asked to continue her Calcium Carbonate and Vitamin D. Repeat labs showed calcium of 7.4 mg/dL. Her symptoms had resolved.

Clinical Lesson: Potential serious hypocalcaemia in eucalcaemic individuals may not be as rare as previously thought. This is the first known case of Denosumab induced severe hypocalcaemia in an otherwise eucalcaemic individual. Denosumab is a potent Osteoclast inhibitor approved for the treatment of Postmenopausal Osteoporosis. Our patient had risk factors for hypocalcaemia including Vitamin D deficiency gastrectomy and malabsorption. We believe that our patient maintained eucalcaemia through the resorptive action of parathyroid hormone on her osteoclasts. Administering a potent Osteoclast inhibitor such as Denosumab might have deprived our patient from the only resorptive mechanism maintaining her serum calcium close to normal. In conclusion Physicians should be very cautious when administering Denosumab to individuals at high risk for hypocalcaemia.

Nothing to Disclose: ZMM, SMH, NLK

Pub # P1-234

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title Follow-Up of Bone Mineral Density and Bone Turnover Markers after Parathyroidectomy in Spanish Patients with Primary Hyperparathyroidism: A Five-Year Study

Author String E Ferrero, G Martinez, S Alonso, FJ Garcia-Borda, S Guadalix, OG Villar, C Vargas, J Perea, F Hawkins
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Body

Introduction
Osteoporosis is one of the accepted criteria for parathyroidectomy (PTX) in the management of primary hyperparathyroidism (PHPT). In this study we analyze the evolution of bone mineral density (BMD) and bone turnover markers after successful PTX.

Patients and Methods
53 Spanish patients (mean age 59.9 y, 79.2% women) with surgically-proven PHPT (48 adenomas) were followed for 5 years in our centre. Lumbar spine BMD (LSBMD), serum fasting PTH, 25-OH-vitamin D, serum calcium (SCa), bone-specific alkaline phosphatase (BAP), intact osteocalcin (IOC), urinary type I collagen cross-linked N-telopeptide (NTx), urinary deoxypyridinoline (DPD) and urinary calcium (UCa), were obtained at baseline and yearly thereafter.

Results
LSBMD increased by 1 year after PTX ($6.3 \pm 1.5\%$; $p < 0.01$). LSBMD increased further after the 3rd year reaching $11.3 \pm 2.5\%$, 5 year after PTX ($p < 0.001$ vs baseline). Bone turnover markers consistently decreased after PTX (baseline-5 year after PTX): BAP 27.41 vs 8.14 [$\mu\text{g/l}$] ($p < 0.05$), IOC 19.48 vs 6.24 ng/ml ($p < 0.001$), NTx 110.22 vs 33.74 ng/ml ($p < 0.001$) and DPD 15.66 vs 5.00 nm-mMcr ($p < 0.01$). SCa and UCa levels remained in the normal range over the five years. PTX also resulted in significant decreases in PTH and increases in 25-(OH)-D that continued for at least 5 years after PTX. Percentage changes in LSBMD were positively and significantly correlated with preoperative PTH levels over the study period: 6 months ($r = 0.85$, $p < .015$), 1 year ($r = 0.85$, $p < .025$), and 5 year ($r = 0.88$, $p < .030$).

Conclusion
In PHPT patients, successful PTX produces a significant increase in LSBMD and normalizes serum and urine bone markers (BAP, IOC, NTx and DPD). Our data suggest that preoperative PTH level could predict long-term LSBMD changes after PTX.

Nothing to Disclose: EF, GM, SA, FJG-B, SG, OGV, CV, JP, FH

Pub # P1-235

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title Primary Hyperparathyroidism in Pregnancy: A Case Report and Review of the Literature

Author String G Stamataros
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Body

Introduction
Primary hyperparathyroidism usually presents with asymptomatic hypercalcemia. It is an uncommon condition in women of reproductive age, and its presentation during pregnancy is rare. In this report, a patient presenting with primary hyperparathyroidism in the first trimester is described.

Clinical Case
A 28 year-old woman presented at 11 weeks gestation with nausea, vomiting, and generalized abdominal discomfort of two days duration. She had two previously uncomplicated pregnancies, and denied a personal or family history of hypercalcemia. She did not report nephrolithiasis or any bone disease. Physical examination was negative for a neck mass, and otherwise was normal. Initial laboratory evaluation revealed a serum calcium level 14.1mg/dL [8.6-10.4mg/dL], albumin level 2.9g/dL [3.6-5g/dL], phosphorus 1.8 mg/dL [2.5-4.5mg/dL], and intact PTH level 240 pg/mL [12-88pg/mL]. A neck ultrasound revealed a 2.7cm x 1.2cm heterogeneous nodule posterior to the right lobe of the thyroid gland. Fetal monitoring revealed no fetal distress. Initial treatment included intravenous fluids and calcitonin, resulting in only a modest reduction in serum calcium levels. Her symptoms improved, but her calcium levels remained persistently elevated and greater than 12 mg/dL despite these measures. The patient underwent elective parathyroidectomy resulting in normalization of serum calcium and intact PTH levels. Final pathologic diagnosis was consistent with parathyroid adenoma. The procedure was uncomplicated, and the remainder of her hospitalization was uneventful. The patient eventually delivered a healthy, eucalcemic baby.

Clinical Lesson
Primary hyperparathyroidism in pregnancy is rare, but poses significant risk to both the mother and fetus. Increased awareness may improve early detection and treatment. Traditionally, surgery during the second trimester has been preferred, as organogenesis is complete. Recently, there has been accumulating evidence supporting earlier surgical intervention. In a retrospective series by *Norman et al*, primary hyperparathyroidism in pregnancy was associated with a 3.5 fold increase in miscarriage rates. Importantly, the majority of pregnancy loss occurred in the late first or early second trimester suggesting early intervention is critical. Medical management often fails, and early surgical intervention may improve maternal and fetal outcomes.

[1] Norman J, Politz D, Politz L. Hyperparathyroidism during pregnancy and the effect of rising calcium on pregnancy loss: a call for earlier intervention. *Clinical Endocrinology* 2009; 71:104-109.

Nothing to Disclose: GS

Pub # P1-236

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title The PK/PD Relationship of Calcimimetic KAI-4169 Following Single-Dose Administration to Healthy Young Males

Author String J Shen, A Vick, K Pickthorn, S Huang, G Bell
Seventh Wave, Chesterfield, MO; KAI Pharmaceuticals, South San Francisco, CA

Body **Background:** KAI-4169 is a novel calcimimetic compound that activates the calcium sensing receptor and inhibits secretion of PTH from the parathyroid cells. Along with safety, the PK/PD of KAI-4169, administered as an IV bolus in healthy young males, was characterized in a single-center, randomized, double-blind, single-dose, dose escalation Phase 1 study. Eight subjects were enrolled into each of 4 cohorts (0.5, 2, 5, and 10 mg) and randomized 6:2 to KAI-4169 or placebo. Blood samples were collected at various time points after IV administration for the determination of plasma KAI-4169 and serum intact parathyroid hormone (iPTH). **Objective:** To investigate the quantitative relationship between plasma KAI-4169 and serum iPTH through development of an integrated PK/PD model. **Methods:** Model development consisted of three steps. First, PK data was analyzed independently and a 3-compartment model was selected as the best fit model. Second, the baseline of serum iPTH in placebo treated subjects was modeled to identify a circadian rhythm model. A trigonometric function based model was constructed to capture the serum iPTH circadian rhythmic variation. Third, the PK and the serum iPTH circadian rhythm submodels were integrated into an indirect response PK/PD model to simultaneously fit the plasma KAI-4169 and serum iPTH data. **Results:** The integrated PK/PD model adequately described the data; particularly, at the higher dose levels. The mean (\pm SD) clearance values of plasma KAI-4169 were estimated at 5.30 ± 0.674 , 6.80 ± 0.653 , 8.02 ± 0.936 and 7.87 ± 0.769 L/h for the 0.5, 2, 5 and 10 mg dose levels, respectively. The mean (\pm SD) values of E_{max} were 0.847 ± 0.111 , 0.850 ± 0.0788 , and mean (\pm SD) values of EC_{50} were 22.0 ± 4.80 , 30.2 ± 4.79 [μ]g/L for the 5 and 10 mg dose levels, respectively. The mean (\pm SD) values of volume of distribution at steady state were 113 ± 19.6 , 138 ± 26.0 , 160 ± 21.0 and 152 ± 30.0 L for the 0.5, 2, 5 and 10 mg dose groups, respectively. The large volume of distribution at steady state suggested a significant portion of KAI-4169 was bound to peripheral tissue(s). **Conclusions:** KAI-4169 demonstrated low PK variability and a clear, predictable, and well characterized exposure-response relationship. The PK/PD model adequately characterized the PK/PD relationship of KAI-4169 and may aid in the refinement of dose and regimen selection for future clinical studies.

Sources of Research Support: KAI Pharmaceuticals.

Disclosures: JS: Consultant, Seventh Wave. AV: Consultant, Seventh Wave. KP: Employee, KAI Pharmaceuticals. SH: Employee, KAI Pharmaceuticals. GB: Employee, KAI Pharmaceuticals.

Pub # P1-237

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title Two Cases with Metastatic Calcification in Parathyroid Carcinoma

Author String J Kawashima, E Watanabe, K Sada, S Yamada, S Shimoda, K Tsuruzoe, N Miyamura, E Araki
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Body
Background: Metastatic calcification is known to be a long-term complication that occurs in patients with chronic renal failure accompanied with secondary hyperparathyroidism. However, there have been very few reports describing patients with metastatic calcification resulted from primary hyperparathyroidism due to parathyroid carcinoma (PT-Ca). We report here 2 cases that showed metastatic calcification caused by PT-Ca. Clinical cases: One is a 46-year-old man diagnosed as PT-Ca 12 years ago when he had suffered from renal stones. Although his parathyroid tumor and cervical lymph nodes were surgically resected and radiofrequency ablations to lung metastases were performed, metastases in lung were exacerbated. When he admitted to our hospital, laboratory data showed severe hypercalcemia (16.2 mg/dl) and hypophosphatemia (2.0 mg/dl) with elevated serum intact-PTH (1,180 pg/ml) and serum creatinine (1.31 mg/dl). Serum calcium was controlled by administration of saline, betamethasone and bisphosphonate, but hypercalcemia remained. Computed tomography (CT) showed ground-glass like appearance in lower lobes of his lung and pneumothorax in addition to multiple metastases, although he did not complain about dyspnea then. Hypercalcemia was aggravated and the patient deceased after five months of admission. Autopsy revealed metastatic calcification in multiple tissues, including lung, myocardial fibers and renal tubules. Especially, severe pulmonary calcification was recognized in bilateral lower lobes, which was consistent with the roentgenological findings. The other case is a 74-year-old man diagnosed as PT-Ca when he was examined about muscle weakness. Although a parathyroid tumor in his neck was surgically resected, serum calcium and intact-PTH levels remained elevated (12-18 mg/dl and 2800-3200 pg/ml, respectively) because he had multiple metastases in his lung. The increase of serum creatinine (1.5-2.0 mg/dl) was persistent but did not worsen. CT of the chest showed small infiltrates in lower lobe, which were suspected of aspiration pneumonia. Saline, betamethasone and bisphosphonate were administered, but he was died after three months of admission. Autopsy was performed and revealed metastatic calcification in his lung, which was consistent with the alteration on chest CT.
Conclusion: As metastatic calcifications in those cases were misdiagnosed as pneumonia, it needs to differentiate them when roentgenological alteration is found in the lung with PT-Ca.

Nothing to Disclose: JK, EW, KS, SY, SS, KT, NM, EA

Pub # P1-238

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title Insulin Sensitivity and Bone Mineral Density in Primary Hyperparathyroidism

Author String F Tassone, L Gianotti, F Cesario, C Baffoni, M Pellegrino, CG Croce, G Magro, G Borretta
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Body **Introduction:** Recent data suggest a reciprocal influence between bone and energy metabolism. Mediators have been identified as osteocalcin (OC), which stimulates insulin (IRI) secretion and activity and IRI as bone anabolic factor. Primary hyperparathyroidism (PHPT) stimulates bone turnover, induces osteoporosis and is associated with increased IRI resistance. In PHPT no data exist on the relationship between IRI and bone mineral density (BMD). **Aim:** To evaluate in a series of PHPT patients without known Diabetes Mellitus (DM), the relationship between IRI levels or sensitivity and BMD. **Subjects and Methods:** We studied 267 patients with PHPT (age, mean \pm SD, 58.5 \pm 13.8 yrs; F/M 198/69; BMI 25.2 \pm 4.6 kg/m²; PTH 228.4 \pm 269.3 pg/ml; calcium 11.2 \pm 1.2 mg/dl) without known DM. Fasting blood glucose and IRI as well as BMD at lumbar spine, femur and forearm were measured. IRI sensitivity was assessed by the Quantitative Insulin Sensitivity Check Index (QUICKI). **Results:** In univariate analysis a positive relationship between IRI levels and BMD (R=0.19, p<0.03) or T score (R=0.23, p<0.005) at femoral site was found, but not at lumbar spine nor at forearm. A negative relationship between QUICKI and BMD (R=-0.20, p<0.015) or T score (R=-0.23, p<0.004) at femoral site was found. In multivariate analysis, we found that age (beta= -0.35, p<0.000004), BMI (beta=0.39, p<0.000001), PTH (beta=-0.18, p<0.013) and QUICKI (R=-0.15, p<0.048) exerted an independent effect (R²=0.29) on femoral T score. **Conclusions:** our data show a relationship between insulin levels and/or sensitivity and BMD at femur in PHPT, as found in other diseases associated with insulin resistance. This finding suggest a link between bone and energy metabolism in PHPT. However, the clinical influence of this relationship on the PHPT-related bone damage is to be established.

Nothing to Disclose: FT, LG, FC, CB, MP, CGC, GM, GB

Pub # P1-239

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title Rapid Reversal of Lithium-Induced Hyperparathyroidism: A Case Report

Author String AN Long, H Oktaei, RD Childress, SS Solomon
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Body Background: Lithium induced hyperparathyroidism is a known cause of reversible hypercalcemia. There are no published cases that describe a rapid reversal back to normal parathyroid hormone (PTH) levels within 5 days.

Clinical Case: A 40-year old woman with a history of bipolar disorder treated with 900mg of lithium carbonate per day for 6 years presented with confusion. Initial lab showed an elevated lithium level (2.02 mmol/L, 0.6 to 1.2 mmol/L), corrected calcium (11.2 mg/dL, 8.5 to 10.1 mg/dL), and PTH (362 pg/mL, 14 to 72 pg/mL), low 25OH Vitamin D level (18ng/ml, 30 to 100 ng/ml) and normal phosphorus (3.9 mg/dL, 2.5 to 4.9 mg/dL). Lithium was held, IV fluids initiated, and hemodialysis was performed with rapid improvement in mental status. Daily calcium levels showed corrected calcium return to normal (9.7 mg/dL, 8.5 to 10.1 mg/dL) within 2 days. Repeat PTH level 5 days after admission also showed normalization (31.2 pg/dL, 14 to 72 pg/dL) with clearance of lithium (<0.2 mmol/L, 0.6 to 1.2 mmol/L).

Discussion: Lithium is known to block calcium influx into cells by competitively inhibiting calcium transport across cell membranes. Higher extracellular calcium levels are needed to raise intracellular calcium levels, suggesting that lithium interferes with the action of the parathyroid calcium-sensing receptor and inositol phosphate metabolism signaling, thereby increasing the set-point for PTH secretion. Hypercalcemia can occur within one day of treatment, and the risk of hyperparathyroid induced hypercalcemia increases with duration of lithium treatment. The typical resolution of hyperparathyroid induced hypercalcemia in patients treated with lithium for over 5 years is up to 8.5 weeks. However, the fastest reported case of PTH recovery to a normal range is 4 weeks. The treatment of choice for severe lithium toxicity is hemodialysis, but the indications for hemodialysis remain controversial. Hemodialysis is suggested in patients with an absolute lithium level above 4 mmol/L or 2.5 mmol/L with impaired mental status. This patient had documented rapid improvement in lithium, calcium, PTH, and mental status after IV fluids and hemodialysis.

Conclusion: Patients with lithium toxicity are at risk of hyperparathyroid induced hypercalcemia, and the rapid reversal of calcium and PTH elevation can occur within a 5 day period with the use of IV fluids and hemodialysis.

Nothing to Disclose: ANL, HO, RDC, SSS

Pub # P1-240

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title Increased Circulating Concentrations of PTH 1-84 Are Related to 6-Year Mortality in Patients with Congestive Heart Failure

Author String D Gruson, T Lepoutre, S Ahn, M Rousseau
Cliniques Universitaires Saint Luc, Brussels, Belgium; Cliniques Universitaires Saint Luc, Brussels, Belgium

Body **BACKGROUND:** Parathyroid hormone (PTH) is related to left ventricular hypertrophy in patients with end stage renal disease and secondary hyperparathyroidism. Furthermore, PTH is associated with worse prognosis in patients with overt HF and has an in vitro a hypertrophic effect on cardiomyocytes. The aim of the present study was to evaluate the relation between the circulating levels of bioactive PTH 1-84 and mortality in severe HF patients.

METHODS: PTH 1-84 concentrations were determined in 76 patients with severe HF (20 women and 56 men; mean age of 68 years and mean ejection fraction of 23%) with the LIAISON third generation PTH assay (DiaSorin), a two-site automated sandwich immunoassay. The endpoint was defined as cardiac mortality, and the study participants were followed up for 6 years.

RESULTS: Of the 76 patients enrolled, 41 died and 35 survived during follow-up. At baseline the mean PTH 1-84 concentration was significantly increased in the patients who died than in those who survived (41.7 vs 27.0 pg/mL, P = 0.01). Kaplan-Meier curve analyses showed that mortality was increased in the the second tertile (15 deaths) and third tertile (18 deaths) in comparison to the first tertile (8 deaths; P= 0.03). The risk stratification power of this assay was more efficient than with the second generation PTH assay.

CONCLUSIONS: Our results have demonstrated for the first time that bioactive PTH 1-84 levels are significantly higher in the heart failure patients with a worse prognosis. Furthermore, this increase of PTH 1-84 was strongly associated with the sixth year cardiac mortality in these patients.

Nothing to Disclose: DG, TL, SA, MR

Pub # P1-241

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title A Review of the Utility of Ultrasonography for Preoperative Localization of Parathyroid Adenomas

Author String RF Ennis, AC Golding
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Body **OBJECTIVE:** To review one medical center's experience with the use of ultrasound (US) and US guided FNA for preoperative localization of parathyroid adenomas.
BACKGROUND: A solitary adenoma is the cause of primary hyperparathyroidism in close to 90% of cases. The traditional surgical approach to primary hyperparathyroidism involves bilateral neck exploration. Effective preoperative localization permits minimally invasive parathyroidectomy which is associated with reduced morbidity, operating time, and expense. US and Tc 99m sestamibi are the most commonly used imaging modalities for preoperative localization.
DATA: Over the past 3 years, a single endocrinologist at our institution has evaluated 185 patients referred for primary hyperparathyroidism. 149 (81%) of the patients were female. The mean calcium was 11.2 mg/dL (range 9.7-14.1, normal 8.5-10.5) and mean PTH was 143 pg/mL (range 42-978, normal 15-65). A lesion that was considered to be consistent with a parathyroid adenoma was noted in 166 (90%) of referred patients. Due to either convincing sonographic characteristics or patient preference, 33 of those patients did not undergo FNA. Twenty of those patients have not yet had surgery. Thirteen have, and surgical concordance with US localization was noted in 12 (92%). Among the 166 patients with possible adenomas on US, 133 did have FNA-PTH. FNA was negative (FNA PTH less than serum PTH) in 14 (11%), but it was positive in 119 patients. Twenty-seven of the patients with a positive FNA are either awaiting surgery or opted for conservative management, and the remainder have had surgery- typically a directed, or minimally invasive, parathyroidectomy. Of the 92 patients treated surgically, concordance between preoperative localization and surgical findings was observed in 91 (99%). One of the 91 had a second contralateral adenoma not noted on ultrasound. From the initial group of 185 patients, 119 have had successful surgical management thus far, and 103 (89%) were identified by ultrasound +/- FNA.
CONCLUSION: This retrospective analysis of one medical center's experience with US and US guided FNA for localization of parathyroid adenomas indicates that these techniques have facilitated many successful and minimally invasive parathyroidectomies. As the gold standard (successful surgical management) has not yet been completed in all referred patients, it is not possible to calculate the sensitivity and specificity of these techniques.

Nothing to Disclose: RFE, ACG

Pub # P1-242

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title PTH-Mediated Hypercalcemia in a Patient with Poorly Differentiated Neuroendocrine Uterine Carcinoma

Author String T Barrera, R Nalini, A Gutierrez, J Suliburk, M Anderson, P Pinto
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Body Background: Hypercalcemia can be PTH mediated (primary hyperparathyroidism) or non-PTH mediated (malignancy). Here we describe an unusual case of hypercalcemia mediated by PTH, the source of which is unclear.
Clinical Case: A 40 year old female with newly diagnosed metastatic poorly differentiated carcinoma of uterine origin presented with weakness, confusion, abdominal pain and extreme thirst. She had noted progressive fatigue and a 45 pound weight loss over few months. Her exam was notable for cachexia, dehydration, altered mentation, and abdominal tenderness. Laboratory investigation revealed sodium 130 mmol/L (136-145), calcium 20.3 mg/dL (8.5-10.1), creatinine 2.3 mg/dL (0.6-1.3), phosphorus 3.7 mg/dL (2.5-4.9), albumin 3.2 g/dL (3.4-5.0), iPTH level 345 pg/mL (10-69), PTHrP <0.03 pmol/L (0-1.5), urine calcium 449 mg/24 hr (100-300), ionized calcium 2.65 mmol/L (1.15-1.29), and 25-hydroxyvitamin D <7.0 ng/mL (32-100). Patient was treated with intravenous hydration, furosemide, zoledronic acid and calcitonin with marked improvement in her calcium. Parathyroid sestamibi scan, parathyroid focused ultrasound, and CT neck failed to localize an adenoma. Selective venous sampling of the neck, IVC and bilateral iliac veins for PTH demonstrated a 20% gradient favoring an intra-abdominal source. A total abdominal hysterectomy and bilateral salpingo-oophorectomy was performed and bulky unresectable periaortic lymphadenopathy was encountered. Pathology revealed a poorly differentiated carcinoma of the uterus with focal neuroendocrine differentiation. The possibility of ectopic PTH production was raised but tissue immunohistochemistry for PTH was inconclusive. One week later, calcium was again elevated requiring medical therapy. Neck exploration for possible parathyroid adenoma(s) was deferred so that patient could begin chemotherapy. Two weeks after chemotherapy, serum calcium normalized. Despite chemotherapy the patient unfortunately died from her advanced malignancy.
Conclusion: Our case represents an unusual scenario of PTH mediated hypercalcemia in malignancy. The source of the PTH remains unclear. The findings in this case, including negative neck imaging, the selective venous sampling and the neuroendocrine nature of the tumor support the diagnosis of ectopic PTH secretion by the cancer. However, primary hyperparathyroidism is not excluded as the immunohistochemistry was not conclusive and neck exploration was not done.

Nothing to Disclose: TB, RN, AG, JS, MA, PP

Pub # P1-243

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title Case Series of Hyperparathyroidism Associated with Radioactive Iodine Therapy

Author String EK Koh, D Kim, X Wang
Abington Memorial Hospital, Abington, PA; UMDNJ, New Brunswick, NJ; UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ

Body **Introduction:** Radioactive iodine (RAI) treatment has been suggested to cause primary hyperparathyroidism (HPT). We describe a series of patients with HPT and a history of RAI for Graves' disease.
Clinical Cases: Case 1: A 46 year-old woman with a history of Graves' disease, treated with RAI therapy 10 years ago, presented for evaluation of hypercalcemia with concurrent elevation of PTH level. She complained of excessive fatigue, but denied any other symptom associated with hypercalcemia. Laboratory evaluation revealed total serum calcium level of 11.4mg/dl (ref range: 8.6 -10.3), intact PTH of 119 pg/ml (ref: 12-72) and 1,25-OH-Vitamin D level of 84.8 ng/ml (ref: 15-55). Other laboratory findings including serum phosphatase level and 25-OH-Vitamin D level were unremarkable. Imaging studies confirmed the diagnosis of parathyroid adenoma, and a surgical resection of parathyroid adenoma was planned.
Case 2: A 36 year-old woman with a history of hypothyroidism secondary to RAI therapy 14 years ago for Graves' disease presented for routine follow up. She was noted to have asymptomatic hypercalcemia with elevated PTH level. Laboratory investigation revealed total serum calcium level of 10.9 and iPTH of 121, whereas other laboratory findings were within the reference range. She was eventually diagnosed with HPT and treated with resection of parathyroid adenoma.
Discussion: Several case reports have suggested the possible link between RAI therapy and subsequent development of HPT. The causal relationship between RAI therapy and HPT is further supported by several animal experiments. However, the underlying mechanism remains unclear. It is hypothesized that a small portion (~10%) of gamma irradiation from RAI taken up by thyroid tissue can affect susceptible adjacent parathyroid glands, which might contribute a consequent development of a parathyroid tumor. In a case series study of 47 patients, the majority of patients were treated for benign thyroid diseases, and the average time between RAI therapy and the diagnosis of HPT was found to be ~ 13.5 years. In addition, pathology of parathyroid glands was predominantly parathyroid adenoma. Our patients reported in this case series are consistent with the characteristics of previous findings.
Conclusion: Anecdotal evidences suggested that RAI therapy for Graves' disease might induce development of HPT. Clinicians should consider monitoring serum calcium level after RAI therapy.

Nothing to Disclose: EKK, DK, XW

Pub # P1-244

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title Chloride:Phosphate Ratio as Screening for Primary Hyperparathyroidism in Calcium Stone Formers

Author String AL Chu, BJ Matawaran, D Bolong, LB Mercado-Asis
University of Santo Tomas Hospital, Manila, Philippines; University of Santo Tomas Hospital, Manila, Philippines

Body

CONTEXT: In 15% of patients with nephrolithiasis, it is due to an underlying disorder, the most common of which is primary hyperparathyroidism. Its diagnosis is based on the level of serum calcium and serum parathyroid hormone (PTH). With the introduction of PTH assay, this test was frequently ordered in the evaluation with only a few were found positive. This study evaluates the use of the chloride phosphate ratio in patients with nephrolithiasis as a screening tool.

OBJECTIVE: To determine the sensitivity and specificity of the chloride phosphate ratio in the screening for primary hyperparathyroidism in calcium stone formers.

METHOD: Forty six subjects with nephrolithiasis were included. Clinical and biochemical profiles of each patient were reviewed. Random blood was extracted for serum chloride and phosphate and compared with ionized calcium and intact PTH as the reference standard. Baseline characteristics were compared using the Independent t-test, Chi Square Test and Fishers Exact Test. The sensitivity, specificity, and likelihood ratios of the test against reference standard were computed, as well as the receivers' operator curves.

RESULTS: Among the 46 subjects, 17 were biochemically positive for primary hyperparathyroidism (hyperparathyroid group). Bilateral nephrolithiasis and nephrocalcinosis were relatively more common in the hyperparathyroid group. There was a statistical difference between the hyperparathyroid group and non-hyperparathyroid group in the levels of creatinine, phosphate, ionized calcium and intact PTH level, as well as the chloride phosphate ratio. The chloride phosphate ratio of ≥ 33 has a sensitivity of 70.6%, specificity of 62.1%, positive likelihood ratio of 1.86 and negative likelihood ratio of 0.473 for the detection of primary hyperparathyroidism in calcium stone formers. Using the ROC analysis, the best cut-off is at 32.4 with an area under the curve of 0.647 while a cut-off of 28 has a sensitivity of 94%.

CONCLUSION: In the screening of primary hyperparathyroidism among patients with nephrolithiasis, a chloride phosphate ratio of 33 has a sensitivity of 70% and specificity of 62%. Adjusted cut-off of 28 has a higher sensitivity of 94% providing higher evidence against primary hyperparathyroidism.

Nothing to Disclose: ALC, BJM, DB, LBM-A

Pub # P1-245

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title Diaphyseal Femoral Fracture Associated with Hyperparathyroidism and Brown Tumor with Sclerotic Bone Response to Parathyroidectomy

Author String FM Alshahrani, DL Kendler, K Khashogi
University of British Columbia, Vancouver, Canada

Body Brown tumor is a rare complication of longstanding hyperparathyroidism. The pathology is well-defined but the radiologic appearance has previously been described as lytic with rare reports of sclerotic lesions after therapy. Brown tumors can affect the mandible, maxilla, ribs, clavicles and rarely long bones. When multiple, these lesions may mimic malignancy, metastatic to bone. We present a 35 year old East Indian woman who presented with a pathological, atraumatic right femoral diaphyseal fracture. Curettings of the femoral shaft at the time of surgical nailing showed [ldquo]brown tumor of bone[rdquo] but did not show malignant change. Subsequent isotope bone scan showed multiple skeletal areas of uptake in proximal femur, and T12 vertebra. Imaging studies showed lytic lesions at these locations. Parathyroidectomy surgery yielded a 1.2cm solitary parathyroid adenoma. There was postoperative normalization of hypercalcemia and elevated PTH levels. Subsequent radiographs and DXA BMD studies showed an intense blastic response at the sites of prior bone scan uptake and radiographic lytic lesions. We describe an atypical diaphyseal femoral fracture associated with hyperparathyroidism and solitary parathyroid adenoma. Surgical cure of her primary hyperparathyroidism resulted in an atypical blastic appearance of the previously lytic lesions. These abnormalities might be easily confused with bisphosphonate related atypical femoral fracture or bone metastatic disease.

1. Yilmazlar S, Arslan E, Aksoy K, et al. Sellar parasellar brown tumor: case report & review of literature. *Skull Base*. 2004; 14:163- 168.
2. Keyser JS, Postma GN: Brown tumor of the mandible. *Am J otolaryngol* 1996, 17:407- 410.
3. Delbridge LW, Younes NA, Guinea AI, Reeve TS, Clifton-Bligh P, Robinson BG. Surgery for primary hyperparathyroidism 1962-1996: indications and outcomes. *Med J Aust* 1998; 168: 153-6.
4. Fitzgerald P: *Endocrinology in current medical diagnosis & treatment*. 39th edition. Edited by Tierney Lm McPhee SJ, Papadakis MA. Stamford, CT: Appleton & Lange, 2000:1118-1121.
5. Nadial Y, Waleed S, Shukri S. Brown tumor of the femur associated with double parathyroid adenomas. *Saudi Med J* 2004; Vol.25(11):1701-1703.
6. Deshmukh R. G, Alsagoff S. A. L, Krishnan S, Dhillon K. S, Khir A. S. M. Primary hyperparathyroidism presenting with pathological fracture. *J R Coll Surg Edinb* 1998; 43:424-427.
7. Ming-Chun H, Jih-Yang K, Hock-Liew E. Pathologic fracture of the distal femur in osteitis fibrosa cystica simulating metastatic disease. *Arch Orthop Trauma Surg* (2004) 124:498-501.
8. Hussain A, Mahmood H, Geddoa E, Hamish M, Reda AH. Parathyroid adenoma and hypercalcemia in a patient presenting with multiple pathological fracture of the long bone. *Int J Surg*. 2008 Dec;6 (6):465-8.
9. Suchecki G, Szudejko B. diagnostic difficulties in primary hyperparathyroidism: a case report. *Chir Narzadow Ruchu Ortop Pol*.1999;64(1):81-3.
10. Yalcin MB, Hiz M, Unlu MC, Dervisoglu S, Kanberoglu K, Bilge I, et al. A case of brown tumor mimicking fibrous dysplasia in a patient with chronic renal failure. *Acta Orthop Traumatol Turc* 2008; 42 :296-301.
11. Pérez JA, López B, Valdivia P, Norambuena L. Pathologic fracture of the femur due to primary hyperparathyroidism. *Rev Med Chil*. 1989 Sep;117(9):1018-22.
12. Goerlich R. left spontaneous femoral fracture in primary hyperparathyroidism and postgastrectomy bone disease following a Billroth II stomach resection. *Chirurg* 1983 Sep;54(9):600-4.
13. Nezh M, Sabri B, Engin G, Sukru B, Oner S, Nil C, Mediha A. brown tumors mimicking bone metastases. *J Natl Med Assoc*. 2006 June;98(6):950-953.
14. Al-Homrany M, Grillo IA, Al-Ghamdi B, El-Tahir M, Malatani TS, Khan MR. Diffuse pulmonary calcinosis & multiple soft tissue calcification in renal failure patient with pathological femoral fracture. *Indian J Chest Dis Allied Sci*.1998 Jul-Sep;40(3):205-11.
15. Diana M, Leonard R, Frank G. Hyperparathyroid crisis in a pediatric patient. *International Journal of Pediatric Otorhinolaryngology* (2004) 68,237-241.
16. Albright F, Aub JC, Bauer W. Hyperparathyroidism, a common & polymorphic condition as illustrated by seventeen proved cases from one clinic. *JAMA*. 1934;102:1276-87.
17. Silverberg SJ, Shane E, Dempster DW, Bilezikian JP. 1999 The effects of Vitamin D insufficiency in patients with primary hyperparathyroidism. *Am J Med* 107:561-567.
18. Grey A, Lucas J, Horne A, Gamble G, Davidson JS, Reid IR. Vitamin D repletion in patients with primary hyperparathyroidism and coexistent vitamin D insufficiency. *J Clin Endocrinol Metab* (2005);90:2122-2126.
19. Murphey MD, Sartoris DJ, Quale JL, Pathria MN, Martin NL. Musculoskeletal manifestations of chronic renal insufficiency. *Radiographics*. 1993 Mar;13(2):357-79.

Nothing to Disclose: FMA, DLK, KK

Pub # P1-246

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title Causes of Hypoparathyroidism in 38 Patients

Author String A Ryndak-Swiercz, E Proust-Lemoine, C Cardot-Bauters, N Porchet, J-L Wemeau H[ocirc]pital Huriez, Lille, France; Centre de Biologie, Lille, France

Body Hypoparathyroidism is a rare endocrine disorder characterized by hypocalcemia and reduced concentrations of parathyroid hormone (PTH). Causes of hypoparathyroidism may be autoimmune, postsurgical or genetic but etiology remains unidentified in many cases. We studied clinical, biological and genetic features of hypoparathyroidism and proposed relevant items to help etiological diagnosis. We investigated patients with hypoparathyroidism in the North of France. We excluded postsurgical causes. Thirty-eight patients with hypoparathyroidism were reported. Seven different causes were found. Autoimmune hypoparathyroidism was diagnosed in 9 cases, DiGeorge syndrome in 7 cases, HDR (hypoparathyroidism, deafness and renal dysplasia) syndrome in 2 cases, activating mutations of calcium sensing receptor (CaSR) gene in 3 cases. One case of syndromic hypoparathyroidism was due to a 10p deletion and another case occurred in ring chromosome 18 abnormality. Hypoparathyroidism caused by hypomagnesemia was found in 1 case. Etiology of hypoparathyroidism remained unknown in 14 patients. There were no striking differences between the groups in ages of revelation, calcemia and PTH levels. Autoimmune hypoparathyroidism was transient in 2 patients who harbored anti-CaSR autoantibodies. A functional role of anti-CaSR antibodies was evocated. Two other patients had undetectable PTH levels indicating parathyroid irreversible damage. Persistent secretion of PTH in subjects with DiGeorge syndrome suggested parathyroid hypoplasia as a mechanism of hypoparathyroidism. Latent hypoparathyroidisms were frequent in this population. Varying phenotypes of HDR syndrome were seen in the 3 members of a same family. The patient with chromosomal 10p deletion showed signs of HDR syndrome (sensorineural deafness) associated with dysmorphic features and retardation. Combination of hypoparathyroidism, granulomatosis and recurrent pneumonia was observed in the patient with mosaic ring chromosome 18. Among the 14 undetermined cases, etiological diagnosis was suspected in half of them but investigations could not be completed. A summary table of causes and relevant features of hypoparathyroidism was proposed. Causes of hypoparathyroidism are various and difficult to identify. Meticulous clinical examination associated to biological, immunological and molecular explorations may be helpful in etiological diagnosis.

Nothing to Disclose: AR-S, EP-L, CC-B, NP, J-LW

Pub # P1-247

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title A Mysterious Parathyroid Adenoma

Author String S Faiz, JW Leidy, TFM Saleem
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Body **Background:** Ectopic parathyroid adenomas have been found in the retroesophageal and retropharyngeal spaces, tracheoesophageal groove, mediastinum, aortic arch, and cervical carotid sheath.
Case Presentation: A 61 year old man was evaluated for chronic mild hypercalcemia, osteopenia, chronic kidney disease stage 3 and declining higher mental function. Laboratory values showed calcium 11.2 mg/dl (8.4-10.7), phosphorus 1.8 mg/dl (2.2-4.7), intact PTH 153 pg/ml (15-63), creatinine 2.4 mg/dl (0.5-1.3), GFR 34 and calcium to creatinine clearance ratio 0.01.
He was diagnosed with primary hyperparathyroidism. Dual phase Tc^{99m} sestamibi scintigraphic planar images showed increased uptake, inferior to left thyroid lobe. Hypercalcemia and hyperparathyroidism persisted despite surgical exploration of the neck and thorax on two separate occasions including left thyroidectomy by the local surgeon.
The diagnosis of primary hyperparathyroidism was reconsidered. Further evaluation showed normal PTH-RP, SPEP, UPEP, PSA and TSH. CT scan and MRI of neck did not show a parathyroid adenoma. Repeat nuclear scan demonstrated increased left inferior uptake. During the third exploration at a tertiary care center, thymectomy was performed but neither a parathyroid adenoma was found nor the intra-operative PTH level declined.
The surgeon started looking at rare ectopic locations. Finally, he found and resected a parathyroid adenoma (1.7 gram and 2.5 x 1.7 x 0.7 cm) in the thoracic part of carotid sheath just below left clavicle. Intra-operative PTH dropped from 272 pg/ml to 30 pg/ml.
Discussion:
Ectopic location is a common reason for unsuccessful neck exploration for a parathyroid adenoma. The finding of a parathyroid adenoma in the infraclavicular portion of the carotid sheath is rare and has not been reported previously to our knowledge. Subsequent review of the previous MRI revealed the adenoma in its unusual location. Localization by an experienced parathyroid surgeon is very important for successful parathyroid surgery even in the modern era of pre- and intra-operative aids.

Nothing to Disclose: SF, JW, TFMS

Pub # P1-248

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title Hypercalcemia Due to an Intrathyroidal Parathyroid Adenoma

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Body

Background
An infrequent cause of primary hyperparathyroidism is a parathyroid adenoma in an intrathyroidal location.

Clinical Case
A 44-year-old female presented with a 2-year history of a right-sided anterior neck mass. Initial complaints were of generalized weakness, weight loss, constipation and bilateral knee pain. Thyroid function tests showed hypothyroidism with TSH 4.0 uIU/mL (0.4-3.8) and free T4 0.07 pmol/L (11-24). Neck ultrasound revealed an enlarged cystic right thyroid lobe and fine needle aspiration biopsy of the mass yielded colloid material. Right thyroid lobectomy was then recommended.

Perioperatively, there was an incidental finding of an elevated serum calcium at 3.19 mmol/L (2.12-2.52) which led to further investigation. Intact PTH was found to be elevated at 1,487 pg/mL (12-65). A Tc-99m sestamibi parathyroid scan showed increased uptake in the right thyroid lobe with accumulation in the right inferior pole. Thyroid scintigraphy with radioiodine (¹³¹I) was also done showing a hypofunctioning mass in the right thyroid lobe. Other diagnostics included bone densitometry which showed osteoporosis with a T-score of -3.5.

After right thyroid lobectomy, exploration of the right side of the neck did not reveal any mass that correlated with the findings on sestamibi parathyroid scan, although a nodule was palpated in the inferior pole of the excised right thyroid lobe. Several structures resembling parathyroid tissue were also excised and sent for frozen section, but none of these were identified to be parathyroid tissue. Final histologic examination of the right thyroid lobe showed the presence of chronic lymphocytic thyroiditis and a 5 x 4 x 3 cm parathyroid adenoma surrounded by a pseudocapsule of thyroid tissue. Post-operative serum calcium was normal at 2.5 mmol/L.

Primary hyperparathyroidism from ectopically located parathyroid glands occurs in close to 9% of all cases, with the incidence of an intrathyroidal location reported at 1-18%. This is associated with larger glands, higher calcium levels and a higher frequency of bone disease.

Conclusion
In the setting of hyperparathyroidism, an intrathyroidal parathyroid adenoma should be considered in the presence of a thyroid nodule and negative surgical neck exploration.

Nothing to Disclose: MTC-C, JAQ

Pub # P1-249
Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)
Title Referrals for Surgery in Patients with Asymptomatic Primary Hyperparathyroidism: The Impact of the Third International Workshop on Clinical Practice
Author String M Vidyarthi, Z Khatami, R Grant, S ElNaas, K Nikookam, EM Casey, B Hossain, E Marouf, FM Nkonge, ND Stojanovic
Queen's Hospital, Romford, UK; Queen's Hospital, Romford, UK; Queen's Hospital, Romford, UK; King George Hospital, Goodmayes, UK

Body **BACKGROUND**
Osteoporosis is a criterion for operative management of patients with primary hyperparathyroidism (PHPT). Most patients with PHPT are now diagnosed at the asymptomatic stage of the disease. Bone mineral density (BMD) is therefore an integral part of the assessment for all patients with asymptomatic PHPT. As parathyroid hormone (PTH) is catabolic for cortical bone, the distal third of the radius (i.e. the wrist) is a convenient site for estimation of BMD. The current guidelines advocate surgery as a definitive treatment for PHPT in asymptomatic patients with established osteoporosis at any site, including the distal radius.

AIMS

The change in the clinical profile of PHPT from overtly symptomatic to asymptomatic has necessitated a paradigm shift in the decisions leading to parathyroid surgery. In this cohort study we examine the effects of the recommendations of the Third International Workshop on the Management of Asymptomatic Primary Hyperparathyroidism (2009) on the referrals for parathyroid surgery made on the basis of osteoporosis from our endocrine clinic.

METHODS

We carried out a retrospective survey of patients referred for parathyroid surgery on the basis of osteoporosis. These were 21 patients who had BMD measured at the wrist in accordance with current guidelines, which was not routine clinical practice previously. We examine how many additional referrals have been made on the basis of osteoporosis at the wrist alone.

RESULTS

Data are given as mean +/- 1 SD. Of 21 patients referred for surgery, ten (47.61%) were referred on the basis of osteoporosis. Of these ten, two (20%) patients had osteoporosis of the femoral neck, three (30%) had osteoporosis of the spine and the remaining (50%) had osteoporosis of the wrist alone. Previously, these patients would not have been referred for parathyroid surgery on the basis of BMD.

CONCLUSION

The evaluation of skeletal health in patients with PHPT is crucial to their long-term management. The effects of PTH are most easily detectable at the wrist, which is completely composed of cortical bone, as opposed to the spine, which is cancellous bone and the hip, which is a combination of both. In our population, estimation of BMD at the wrist provides an effective method of assessing skeletal health and facilitates the decision for definitive management in patients with asymptomatic PHPT.

Nothing to Disclose: MV, ZK, RG, SE, KN, EMC, BH, EM, FMN, NDS

Pub # P1-250

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title Robotic-Assisted Transaxillary Parathyroidectomy of an Atypical Adenoma

Author String S Ali, M Abdel Khalek1, N Avitabile, A Banka, K Emad
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Body
Introduction:
Primary hyperparathyroidism (PHT) presents a complex surgical and clinical challenge. Difficulties with intraoperative adenoma localization and damage to surrounding structures have long hindered utilizing minimally invasive approaches for parathyroidectomy. The confluence of improved adenoma localization using Sestamibi scan and intraoperative PTH monitoring and the concomitant advent of minimally invasive video technology, have led to fewer complications and greater patient satisfaction
Case ; : 48 year old female who was had PHT due to atypical, encapsulated parathyroid adenoma. Parathyroidectomy with right thyroid lobectomy was performed with robotic-assisted transaxillary surgery to remove adenoma. Intact parathyroid hormone level returned to normal and a greater than 50% drop from baseline was achieved intraoperatively with subsequent long-term cure. There were no complications.
Conclusions: Application of robotic technology for endoscopic parathyroid surgeries could overcome the limitations of conventional endoscopic surgeries in the surgical management of parathyroid adenoma. Robotic-assisted transaxillary surgery is a safe and feasible technique for parathyroidectomy with en bloc resection of the thyroid gland.

Nothing to Disclose: SA, MAK, NA, AB, KE

Pub # P1-251

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title PTH Measurement in the Fine-Needle Aspirates from Cervical Nodules in Primary Hyperparathyroidism

Author String F Bandeira, E Diniz, T Fontenele, R Carvalho
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Body OBJECTIVE: To evaluate the benefits of parathyroid hormone (PTH) measurement in ultrasound-guided (US) fine-needle aspiration (FNA) fluid of nodular lesions in the thyroid bed or suspected of being parathyroidal, in patients with hyperparathyroidism (HPT). METHODS: The patients (Group 1) were 15 women aged 38 to 79 years, while the control group (Group 2) comprised 15 women, aged 32 to 69 years, without hyperparathyroidism and with one or more thyroid nodules diagnosed during routine ultrasound examination. RESULTS: Mean±SD PTH levels in the aspirate of PHPT lesions were 4,919.73±5,124.84 pg/ml (815-22,100 pg/ml), while in the control group they were 10.65±3.49 pg/ml (3.8-16 pg) (p <0.002). In the PHPT group the cytology specimens of 12 patients (80%) were characteristic of parathyroid adenoma, and in 3 patients (20%) the cytology was inconclusive. In the control group, the cytology specimens of 7 patients (46.66%) were characteristic of adenomatous goiter, 4 (26.66%) were colloidal goiter, 1 (6.7%) was lymphocytic thyroiditis, and in 3 patients (20%) they were inconclusive. CONCLUSION: Measurement of PTH in aspiration fluid of cervical nodules has a high accuracy in localizing the parathyroid lesion in patients with PHPT.

Nothing to Disclose: FB, ED, TF, RC

Pub # P1-252

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide I (1:30 PM-3:30 PM)

Title Severe Hypercalcemia Due to Giant Parathyroid Adenoma

Author String A Ghazanfari, JM Bayasi, R Saxena, M Shalpour, MH Horani
Mercy Gilbert Hospital, Gilbert, AZ; Alsham Endocrinology, Chandler, AZ

Body 47 Year old caucasian female with PMH of HTN, GERD presented to the hospital with one month history of generalized fatigue, polyuria and polydypsia. On general exam patient was awake alert Blood pressure 157/78 Heart rate 109
physical exam was remarkable for large left neck mass solid non tender thought to be thyroid mass
Labs revealed Ca of 16.5, albumin 2.8, corrected Ca 17.7, creatinin 2.8, BUN 35
PTH intact 401 pg/ ml
Neck US showed 7.8X 3.8 X3.8 CM possible left thyroid mass solid with internal scattered cystic spaces no microcalcification on Technetium Sestamibi scan there was diffuse prominet activity on early images but on delayed images ther was considerable washout of activity from the mass which was thought to be enlarged thyroid lobe by the nuclear physician
Subsequently patient underwent FNA biobsy and that revealed Prathyroid tissue with negative TTF-1 staining for thyroid tissue. In the mean while pt was treated with IV hydration and Zolendronic Acid , Calcium normalized before Surgery and her creatinine dropped to 1.5.
Eventually patient underwent Surgery and final path revealed Giant 6.0x 4.0x3.0 cm, 11g parathyroid adenoma. No signs of malignancy were observed by both morphological and cell kinetic analyses. post surgery PTH normalized to 16.5 pg/ ml.
Parathyroid adenomas rarely attain huge proportions, and they could be misdiagnosed as thyroid nodules, The prevalence of parathyroid incidentaloma was 0.4% in a large series of patients referred for suspected thyroid nodules (1)
clinicians should be aware of giant parathyroid adenoma in the differential diagnosis of thyroid bed's masses.

Thyroid. 2009 Jul;19(7):743-8.

Nothing to Disclose: AG, JMB, RS, MS, MHH

Pub # P1-253
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)
Title Overweight Reduces Endogenous LH Efficacy and Age Heightens Downregulation of Testicular Sensitivity and LH Potency in Men
Author String PY Takahashi, DM Keenan, JD Veldhuis
Mayo Clinic, Rochester, MN; University of Virginia, Charlottesville, VA

Body Age is associated with gradual declines in free and bioavailable testosterone (T) concentrations and an inferable increase in LH pulse frequency with a reciprocal decrease in incremental LH pulse amplitude. The mechanisms driving these adaptations in the human are not known. We postulated that more rapid and smaller LH pulses in older men may downregulate testicular T responses to endogenous LH. To test this question, 48 healthy men (age 18-72 yr, BMI 20-32 kg/m²) underwent blood sampling every 10 min for 12 hr overnight for concomitant LH and T measurements. The paired LH-T time series were analyzed by a new analytical model of allowable (possible, but not required) downregulation of the LH-T dose-response function comprising efficacy, sensitivity and potency (1,2). The outcomes were subjected to linear and exponential regression analysis. BMI was associated with a linear decrease in LH efficacy (maximally stimulated T secretion rate, P = 0.036, R = -0.303). Age was marked by multiple concurrent regulatory changes: (1) a decrease in gonadal T sensitivity to LH pulses (P = 0.045, R = -0.29); (2) a decrease in endogenous LH potency (reflected by a rise in EC₅₀ for LH, P = 0.05, R = 0.29); (3) an increase in basal (nonpulsatile) T secretion (P < 0.001, R = 0.56); and (4) heightened downregulation of both LH potency [inverse of EC₅₀] (P = 0.023, R = 0.96) and LH sensitivity (P = 0.013, R = 0.97). These data unveil an array of potential pathophysiological mechanisms operating at the testis level in overweight and aging men. A critical clinical question is their possible remediation by restoring a normal LH pulse signal experimentally and/or a normal BMI therapeutically.

(1) Keenan DM, Roelfsema F, Veldhuis JD 2010 Dose-response downregulation within the span of single interpulse intervals. *Am J Physiol* 299:R11-8

(2) Keenan DM, Iranmanesh A, Veldhuis JD 2010 Analytical construct of reversible desensitization of pituitary-testicular signaling: illustrative application in aging. *Am J Physiol Endo Metab* (in press online)

Sources of Research Support: NIH Grant AG31763 and CTSA #RR024150.

Nothing to Disclose: PYT, DMK, JDV

Pub # P1-254

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Elevated Androgens during Puberty Lead to an Increased Neuronal Drive to the Reproductive Axis in the Nonhuman Primate

Author String WK McGee, CV Bishop, A Bahar, CR Pohl, RJ Chang, JC Marshall, FK Pau, RL Stouffer, JL Cameron Oregon National Primate Research Center, Beaverton, OR; Oregon Health & Science University, Portland, OR; Oregon Health & Science University, Portland, OR; Oregon Health & Science University, Portland, OR; University of Pittsburgh, Pittsburgh, PA; University of California, San Diego, La Jolla, CA; University of Virginia Health System, Charlottesville, VA; Oregon National Primate Research Center, Beaverton, OR; University of Pittsburgh, Pittsburgh, PA

Body Polycystic ovary syndrome (PCOS) is a common reproductive disorder, affecting 4-8% of reproductive-aged women worldwide. Studies in young women with PCOS indicate that they have increased pulsatile LH secretion and greater LH responsiveness to GnRH, but it is unclear whether these neuroendocrine changes are causative or are consequences of PCOS. There is growing evidence that slightly elevated levels of circulating androgens may play a causal role in the development of PCOS. In order to test whether a slight elevation in serum androgens leads to an increase in the central drive to the reproductive axis, female rhesus monkeys (n=6/group) were maintained with subcutaneous silastic implants containing either testosterone (T) or cholesterol (C) beginning prepubertally (i.e., at 1 year of age) and lasting into early adulthood (i.e., 5.5 years of age). T-treated animals had T levels that were maintained 3.7±0.2-fold higher than C-treated animals (T-treated: 1.73±0.02 ng/mL; control: 0.50±0.05 ng/mL, p=0.001). Weight gain was similar in both groups until 5.5 years of age when T-treated animals became significantly heavier than controls (T-treated: 5.2±0.1 kg; control: 4.7±0.2 kg; p=0.03), with no difference in food intake. When the animals were five years of age, pulsatile LH secretion was measured for 12 h (1300 h to 0100 h; lights out at 1900 h) during the early follicular phase (D1-3) of a menstrual cycle. T-treated animals had a significantly greater LH pulse frequency than controls (T-treated: 9.7±1.8 pulses; control: 3.7±1.8 pulses; p=0.04), indicating that T-treated animals had an increased central drive to the reproductive axis. Ultrasounds were also performed during the early follicular phase of a separate menstrual cycle to assess ovarian morphology. There were no differences between groups in the numbers of small antral follicles present. There was, however, a trend toward control animals being more likely to have a follicle over 2.0 mm in diameter compared to T-treated animals (p=0.09), with 3/6 controls and no T-treated animals having a follicle of this size. Follicles of this size are more likely to proceed to ovulation in rhesus monkeys. This may indicate that control, but not T-treated animals, are beginning to show signs of normal adult ovarian function. We conclude that a slight elevation in circulating androgens over pubertal development appears to trigger physiological changes in GnRH (LH) secretion that are characteristic of PCOS.

Sources of Research Support: OHSU Center for Women's Health Circle of Giving, pilot funds from RR0163, HD18185, the Eunice Kennedy Shriver NICHD/NIH through cooperative agreements, U54 HD 28934 and U54 HD 12303 as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research, as well as by an R21 grant from NCRR (RR030276). Ms. McGee was supported by a fellowship from the OHSU ARCS Foundation and NIH grant T32 HD07133. Dr. Bahar was supported by fellowship DK007674-13.

Nothing to Disclose: WKM, CVB, AB, CRP, RJC, JCM, FKP, RLS, JLC

Pub # P1-255

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Estradiol Exposure in Postmenopausal Women, but Not Testosterone Supplementation in Older Men, Attenuates GH Feedback-Dependent Suppression of Endogenous Peptide-Driven Pulsatile GH Secretion

Author String JD Veldhuis, D Erickson, JR Wigham, SM Weist, JM Miles, CY Bowers
Mayo Clinic, Rochester, MN; Tulane University Health Sciences Center, New Orleans, LA

Body Pulsatile GH secretion reflects the triple interplay of GHRH and ghrelin (native GHRP) as stimuli and somatostatin as an antagonist. Parsing the precise manner in which these three pivotal peptides interact is difficult in clinical experiments. We use the notion of a 'dual-peptide clamp' to evaluate otherwise unobserved hypothalamic somatostatin outflow to the pituitary. Thereby, we test the hypothesis that estradiol or testosterone administration in healthy older adults (age 50-80 yr) restricts GH's feedback-induced release of somatostatin. The 2-peptide clamp comprised simultaneous continuous combined i.v. infusion of GHRH-1,44 amide and GHRP-2, a synthetic ghrelin analog (both 1 [micro]g/kg/min) compared with saline. In this model, nadir GH values reflect maximal somatostatin outflow evoked by a single i.v. pulse of rhGH (1 [micro]g/kg bolus), whereas peak GH values signify maximal escape, *i.e.* minimal somatostatin antagonism. Each subject (N = 11 women, N = 14 men) underwent 4 infusions (2 after placebo, 2 after sex-steroid repletion). Two-way ANOVA defined $P < 0.001$ for peptide (GHRH/GHRP-2 *vs* saline) effects in both men and women. Testosterone treatment in men did not alter nadir or peak GH values during either saline or 2-peptide infusion ($P > 0.10$). Estrogen repletion in women elevated post-GH-feedback nadir GH concentrations on the saline day by 6-fold ($P = 0.006$). This effect was abolished by combined-peptide infusion ($P = 0.716$). Estradiol also augmented post-feedback peak GH concentrations by 4-fold on the saline day ($P = 0.009$). There was a strong interactive peptide x estrogen effect of $P = 0.009$. This was explained by estrogen's potentiation of peak GH recovery during infusion of saline (under endogenous GHRH/ghrelin drive) but not during infusion of GHRH/GHRP-2 (exogenous GHRH/ghrelin-like drive). The capability of estradiol to elevate nadir and peak GH concentrations under experimentally controlled GH autocrine feedback during saline infusion suggests that this sex steroid may restrict somatostatin release to, or action on, the pituitary gland and/or augment endogenous GHRH and ghrelin outflow. Abolition of these two estrogen effects by exogenous GHRH/GHRP further supports the latter concept. The lack of effect of testosterone compared with estradiol in this paradigm may indicate that a particular threshold level of testosterone-derived estradiol must be achieved in men in order to mute GH autocrine feedback and/or potentiate endogenous GHRH/ghrelin drive.

Sources of Research Support: NIA Grant AG19695 and NIA Grant AG29362 and CTSA #RR024150.

Nothing to Disclose: JDV, DE, JRW, SMW, JMM, CYB

Pub # P1-256

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Expression of Luteinizing Hormone (LH) Gene in the Rat Hypothalamus

Author String SH Lee, H Heo
Sangmyung University, Seoul, Republic of Korea

Body Several lines of evidence indicate that the presence of luteinizing hormone(LH) peptides in the rat brain. So far, studies on the LH in hypothalamus at the transcriptional level has not been performed yet. The present study was undertaken to investigate the expression and regulation of the LH subunit genes in the rat hypothalamus. Immature female rats(4 weeks) were ovariectomized (OVX) and implanted with silastic capsules containing 17 β -estradiol (E₂; OVX+ E₂ group) or vehicle (OVX+Oil group). Two days later, animal received subcutaneously either progesterone (P₄; OVX+E₂+P₄ group) or a vehicle injection 6 hours prior to decapitation. The presence of LH peptides in hypothalamus was examined by radioimmunoassay (RIA) and immunohistochemistry. Amplifications of the cDNA fragments for Cg α , pituitary type LH- β subunit (pLH- β) and testis type LH- β subunit (tLH- β) were demonstrated by RT-PCRs and Southern blot analyses. LH RIA parallelism was shown with the LH standard curve and the competition curve using crude extracts of hypothalami. 3'-RACE revealed that the 3'-end of hypothalamic LH- β transcript is identical to that of the pituitary LH- β transcript. Furthermore, immunostainable Cg α and LH- β were localized in the fornix, mammillothalamic tract, ventomedial hypothalamic nucleus, lateral hypothalamic area, anterior hypothalamic area, premammillary nucleus, lateral mammillary nucleus, mammillary peduncle and arcuate hypothalamic nucleus in the rat hypothalamus. RT-PCR and immunohistochemistry studies revealed that the LH subunit genes were expressed in the hypothalamus from hypophysectomized(HPX) rats, confirming the local production of the LH peptides. The mRNA levels of Cg α , pLH- β and tLH- β in hypothalamus from OVX+O group ($p<0.001$, $p<0.05$ and $p<0.05$, respectively) were significantly higher than those from the intact group, and the levels from OVX+E₂ group ($p<0.01$, $p<0.05$ and $p<0.05$, respectively) were significantly lower than those from the OVX+O group. Administration of P₄ significantly augmented hypothalamic Cg α , pLH- β and tLH- β mRNA levels compared to those from OVX+E₂ group($p<0.05$, $p<0.01$ and $p<0.05$, respectively). The present study demonstrated that local expression of the rat hypothalamic LH subunits, and the expression of hypothalamic LH subunit genes is under the control of ovarian steroidal milieu. The present study suggests that the hypothalamic LH could be participated in the local regulation of hypothalamic neuronal circuits.

Nothing to Disclose: SHL, HH

Pub # P1-257

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Responsiveness to Pulsatile GnRH in Women with Isolated GnRH Deficiency Identifies Additional Pituitary and Ovarian Defects

Author String JM Brown, B Abel, JM Adams, T Alati, ND Shaw, KA Martin, N Pitteloud, SB Seminara, WF Crowley, Jr, CK Welt, JE Hall
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Body **Context:** Replacement with pulsatile GnRH induces ovulation in the majority of women with isolated hypogonadotropic hypogonadism. We hypothesized that evaluation of LH, FSH and estradiol (E₂) responses to GnRH in the first week of treatment would localize additional defects to the pituitary and/or gonad in these GnRH-deficient women as has been seen in GnRH-deficient men¹.
Methods: Pulsatile GnRH was administered intravenously to women with isolated GnRH deficiency (n=41) at a dose (75-100 ng/kg) and frequency shown to result in physiologic follicle development, ovulation and hormone levels. Daily LH, FSH, E₂, & P were measured 45' after a GnRH dose. LH, FSH and E₂ levels over the first 7 days of treatment (q90' pulse frequency) were compared between those who did and did not ovulate within 20 days and correlated with mutations in 11 genes associated with GnRH deficiency.
Results: Thirteen women failed to ovulate in response to initial treatment. LH and FSH levels in the ovulatory group paralleled the normal range by day 2 of treatment and E₂ by day 3. E₂ remained low in anovulatory women. Ten anovulatory women had a deficient pituitary response to initial treatment (LH and/or FSH more than 1 SD below the mean of ovulatory patients). Ovulation was subsequently induced (n=8) when gonadotropins were normalized with prolonged GnRH treatment or an increased dose, consistent with pituitary resistance to GnRH. Three of these patients had *GNRHR* mutations, 1 had a homozygous *KAL1* mutation, and 1 had a mutation in *PROKR2*, while mutations in these genes were not present in the ovulatory patients. Three patients had LH and FSH levels in response to initial treatment that were within or above the range of ovulatory patients, consistent with ovarian resistance. Two ovulated with increased GnRH dose and none had mutations in the known GnRH-associated genes.
Conclusion: Women with isolated GnRH deficiency can exhibit previously unrecognized defects at the pituitary and ovary that are only evident when physiologic GnRH replacement is instituted. Increased dose or duration of GnRH increases the ovulatory rate to 93%. Mutations in *PROKR2* and *KAL1* in women with pituitary resistance suggest a possible role of these genes in pituitary function in addition to their roles in GnRH neuronal development. These phenotypic observations contribute valuable information regarding genes causing isolated GnRH deficiency in women and their potential pituitary and ovarian roles.

(1) Sykiotis GP et al, J Clin Endocrinol Metab 2010; 95:3019.

Sources of Research Support: Eunice Kennedy Shriver NICHD/NIH grant R01-HD-42708 and cooperative agreement [U54 5U54HD028138] as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research.

Nothing to Disclose: JMB, BA, JMA, TA, NDS, KAM, NP, SBS, WFC, CKW, JEH

Pub # P1-258

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title *In Vivo* Study of Pitx2 in the Development of Hypothalamic GnRH Neurons

Author String M-H Quentien, J-P Herman, PJ Gage, J-P Gautron, T Brue
Faculté de Médecine Secteur Nord, Université de la Méditerranée, Marseille, France; University of Michigan Medical School, Ann Arbor, MI; Assistance Publique-H[ocirc]pitaux de Marseille, H[ocirc]pital de la Timone, Marseille, France

Body Hypothalamic gonadotropin-releasing hormone (GnRH) neurons are born in the nasal placode and migrate to the hypothalamus, where they secrete the GnRH into the pituitary portal vessels to induce the release of gonadotropins LH and FSH into the general circulation. Recently, we have found by radioimmuno assay a specific decrease of the GnRH content in the hypothalamus of two months old male and female transgenic mice with invalidation of Pitx2, a paired-like homeodomain transcription factor. No variation of the GHRH and TRH contents was measured in these animals. On one hand, we are determining in males whether our observation can be explained by a decrease in the number of GnRH neurons, resulting from a developmental defect in the migration of these cells. We will examine whether the effect is related to the disappearance of Pitx2 expression in the GnRH neurons themselves, in the neurons and glia involved in guiding their migration or in the neural crest. On the other hand, the decrease of GnRH secretion might be due to a dysregulation of the GnRH expression or secretion itself. In this purpose we are checking the mRNA levels of PITX2 in control and transgenic mice. Altogether, our results show a role for PITX2 in the regulation of the hypothalamo-pituitary axis and also suggest that mutations of PITX2 might be involved in the pathogenesis of a subset of congenital GnRH deficiencies in humans, causing absence or delay of puberty.

Nothing to Disclose: M-HQ, J-PH, PJG, J-PG, TB

Pub # P1-259

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Insulin Action in Kiss1 Neurons and the Regulation of Puberty and Reproduction

Author String X Qiu, AR Dowling, JS Marino, LB Nedorezov, JW Hill
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Body The neuropeptide kisspeptin, coded by gene Kiss1 is necessary for reproduction, fertility, and puberty. Humans and mice with loss-of-function mutation of Kiss1 are infertile due to hypogonadotropic hypogonadism. Insulin receptors and insulin signaling proteins are widely distributed throughout the hypothalamus and play a pivotal role in regulation of energy balance, metabolism, and reproduction. In order to study whether insulin -sensing plays an important role in Kiss1 neuron, we generated Kiss1 neuron specific insulin receptor knockout mice (Kiss1-Cre, IR^{flox/flox} mice). Here, we describe effects on vaginal opening, first estrus cycle, and fertility. Body weight, food intake, and glucose regulation were also examined. These results have important implications for understanding how insulin signaling in Kiss1 neuron may be involved in puberty and reproduction.

Nothing to Disclose: XQ, ARD, JSM, LBN, JWH

Pub # P1-260

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Lack of Functional GABAB Receptors Disrupts Normal Kiss1 mRNA Expression in Neonatal Mice

Author String N Di Giorgio, P Catalano, B Gonzalez, B Bettler, S Rulli, C Libertun, V Lux-Lantos
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University of Basel, Basel, Switzerland; School of Medicine, University of Buenos Aires, Buenos Aires, Argentina

Body Kisspeptin (Kiss1) is one of the most important neuropeptides in charge of the control of gonadotrophic axis controlling GnRH secretion. There are two populations of Kiss1 neurons; one is located in the antero-ventro periventricular nucleus (responsible for the estrogens-induced positive feedback) and the other one in the arcuate nucleus (responsible for the estrogens-induced negative feedback). Estrogens, produced by gonads or locally in the hypothalamus by p450 aromatase (p450aro), are key modulators in Kiss1 mRNA expression. We have previously shown that the absence of functional GABAB receptors (GABAB1KO) alters the hypothalamic-pituitary axis in adult mice (1) and more recently we have discovered that changes already occur in 4-day-old (PND4) mice. GABAB1KO mice have alterations in GnRH and glutamate decarboxylase-67 mRNAs expression in medial basal hypothalamus (MBH), but not in preoptic area-anterior hypothalamus (AH), and also in LH content and serum levels (2). However, these alterations differ from those observed in adulthood, suggesting additional regulations in later stages of development. The aim of this study was to determine whether Kiss1 and p45aro were altered in PND4 GABAB1KO mice compared to WTs. Both sexes, wild-type (WT) and GABAB1KO mice were killed at PND4, their MBH and AH obtained to study Kiss1 and p450aro mRNA expression by RT-PCR and qRT-PCR respectively. Kiss1 expression depends on sex and genotype in MBH (two-way ANOVA: $p < 0.001$). It was sexually dimorphic in WTs; its expression was higher in females than in males ($p < 0.01$). However, this difference was lost in GABAB1KO mice. Moreover, Kiss1 mRNA decreased in GABAB1KO compared to WT females ($p < 0.001$). In contrast, there were no differences between sexes or genotypes in Kiss1 mRNA expression in AH. P450aro mRNA expression was not different between groups either in MBH or in AH. These results show that, in agreement with previous results on GnRH expression, lack of functional GABAB receptors disrupts normal Kiss1 mRNA expression, specifically in the MBH, at an early postnatal stage of development. These alterations in Kiss1 expression are not accompanied by changes in aromatase expression.

1. Catalano PN, Di Giorgio N, Bonaventura MM, Bettler B, Libertun C, Lux-Lantos VA 2010 Lack of functional GABA(B) receptors alters GnRH physiology and sexual dimorphic expression of GnRH and GAD-67 in the brain. *Am J Physiol Endocrinol Metab* 298:E683-E696

2. Di Giorgio N, Catalano PN, Bettler B, Libertun C, Lux-Lantos V, Alterations in the Regulation of the Gonadotrophic Axis In Neonatal GABAB1KO Mice. Abstract, Tenth International Symposium of GnRH, the Hypothalamic-Pituitary Axis in Cancer and Reproduction. Salzburg, Austria, February 6-8, 2011

Sources of Research Support: Grants from CONICET (PIP 363 2010); ANPCyT (BID PICT 2006 N[ordm] 00200 and BID PICT 2007 N[ordm] 01050) and Universidad de Buenos Aires (ME 038).

Nothing to Disclose: NDG, PC, BG, BB, SR, CL, VL-L

Pub # P1-261

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Ontogenesis of the KISS/KISS1R System during Human Hypothalamic Development

Author String F Guimiot, S Dreux, D Chevenne, A-L Delezoide, N De Roux
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Body The KISS/KISS1R system is a key regulator of the gonadotropic axis modulating GnRH secretion and therefore FSH and LH synthesis and secretion. Many studies in animals and humans have been performed to understand the post-natal function of this system, however its development during fetal life is uncharacterized. In this study, we investigated by immunohistochemistry the expression patterns of GnRH, Kisspeptins and KISS-1R during human hypothalamic development and correlated them with FSH and LH foetal plasmatic levels. We found that Kisspeptin expression appeared in the supraoptic nucleus (SO) and ventromedial hypothalamic nucleus (VMH) at 15 weeks of gestation (WG), expanded to the paraventricular nucleus (Pa), infundibulum (INF) and median eminence (ME) from 20 WG to 30 WG and was confined to Pa and SO at birth. KISS-1R appears in SO at 15 WG, expands to the other regions from 20 WG to 30 WG and is confined to Pa and SO at birth. GnRH is detected in the four studied regions from 15 WG to birth. At 30 WG, all components of the system are expressed in the four regions. Simultaneously, FSH and LH fetal plasmatic levels peak around 20-22 WG and decrease until birth both in girls and boys with a sexual dimorphism in girls: who have higher levels of plasmatic gonadotropins. These plasmatic levels are not associated to a decreased number of FSH and LH-expressed cells in pituitary as the relative number of gonadotropic cells regularly increases from 15 to 30 WG then decreases until birth. This study shows that the KISS/KISS1R system is active during fetal life. Furthermore, it indicates that the inactivation of the gonadotropic axis at birth is mainly related to a decrease of gonadotropin secretion, which may be due to an abolished expression of KISS and KISS1R in VMH and ME.

Nothing to Disclose: FG, SD, DC, A-LD, NDR

Pub # P1-262

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Gene Amplification and Up-Regulation of Mammalian STE20-Like Protein Kinase 4 (MST4) Reveal a Novel Candidate Gene in Human Pituitary Tumorigenesis

Author String AJ Knox, MG Edwards, K Kiseljak-Vassiliades, M Xu, KA Michaelis, BK Kleinschmidt-Demasters, KO Lillehei, ME Wierman
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Body Pituitary tumors are the most common brain tumor of which 35% arise from the gonadotrope lineage. The genetic and molecular mechanisms which initiate or promote the growth of gonadotrope tumors are poorly understood. To identify novel gonadotrope tumor candidate genes and pathways we used a combined approach, integrating data from expression microarrays with whole-genome screens for copy number alterations (CNA). Candidate genes with both aberrant expression and overlapping CNAs in tumors were characterized further. A microarray screen (HumanU133Plus2.0) compared the expression profiles of 14 gonadotrope tumors versus 9 normal pituitaries from autopsy using an ANOVA model to identify significantly altered genes. To detect gene amplifications and deletions, a microarray-based copy number screen was performed on Genome-Wide Human SNP 6.0 Arrays in 10 gonadotrope tumors. CNAs were assessed using 794 HapMap samples as the baseline genomes and segmentation analysis revealed 110 amplifications and 336 deletions across all tumors. MST4 mRNA expression was upregulated by 6.3-fold in tumors compared to normal pituitary and was found to be located within a 390 kb amplification at Xq26.2 in one gonadotrope tumor sample from a female patient. MST4 is a novel serine-threonine kinase, whose upstream regulators are poorly defined, that is upregulated in prostate, pancreatic, liver, and metastatic colon cancer where it may direct increased anchorage independent growth, *in vitro* proliferation, cell migration, and *in vivo* tumorigenesis. Analysis of prior published expression arrays suggest that MST4 mRNA is expressed in prolactin, growth hormone, and ACTH tumors at levels comparable to normal pituitary in contrast to the upregulation in gonadotrope tumors. RTPCR validation of MST4 mRNAs showed a 2-fold increased expression in gonadotrope tumors compared to normal pituitary and demonstrated the highest MST4 mRNA levels in the tumor sample carrying the MST4 amplification. Immunoblots confirmed that MST4 protein levels are also increased in gonadotrope tumors and low or undetectable in normal pituitary. A second predicted gene, LOC286467, was also within the Xq26.2 amplification; however, the transcript was undetectable in normal or tumor. In summary, MST4 is a promising candidate for uncovering novel mechanisms and pathways underlying pituitary tumorigenesis and is a potential therapeutic target.

Sources of Research Support: VA Merit Review to MEW, UCD Cancer Center and Genomics Core.

Nothing to Disclose: AJK, MGE, KK-V, MX, KAM, BKK-D, KOL, MEW

Pub # P1-263

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Repression of GADD45 β in Human Gonadotrope Pituitary Tumors: Role in Proliferation, Survival and Tumorigenesis

Author String KA Michaelis, AJ Knox, M Xu, K Kiseljak-Vassiliades, MG Edwards, BK Kleinschmidt-DeMasters, KO Lillehei, M Geraci, ME Wierman
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Body The genetic and molecular mechanisms underlying the pathogenesis and/or progression of gonadotrope pituitary tumors are under active investigation. Microarray expression and bioinformatic analysis was performed comparing 14 human gonadotrope tumors and nine normal pituitaries. ANOVA was used to generate p-values for present genes (38,932 of 54,675 transcripts on the Affymetrix U133 2.0 array) with a false discovery rate (FDR) of 5% to control for multiple testing. Differentially expressed genes were analyzed using Ingenuity Pathway Analysis software ($P < 0.05$) in tumor vs normal. Multiple pathways were identified including components downstream of p53 ($p = 1 \times 10^{-4}$). GADD45 β was repressed 68-fold in tumor compared to normal with no change in GADD45 α and a 44-fold repression of GADD45 γ as previously reported. Loss of GADD45 β protein was confirmed in gonadotrope tumors compared to normal pituitaries. The GADD45 β promoter is methylated in liver and non-small cell lung cancer to block its expression. In contrast to these results and GADD45 γ methylation in pituitary tumors, bisulfite sequencing showed that downregulation of GADD45 β was not due to promoter methylation in any of the 12 tumors examined. Stable overexpression of GADD45 β (14-fold) in mouse L β T2 gonadotrope cells revealed decreased cell proliferation (30%) over 9 days by an MTS assay ($p = 0.0005$). GADD45 β overexpression repressed colony formation in soft agar 74% compared to vector controls at 21 days (153 ± 7 versus 590 ± 30 , respectively, $p = 0.0007$). Serum deprivation for 72h increased rates of apoptosis 2-fold in GADD45 β transfectants compared to controls. Prior studies suggested that upregulation of GADD45 β induces apoptosis via p38 MAPK signaling. Conversely, in pituitary cells and in human pituitary tumors, p38 MAPK activity is high. Coincubation of gonadotrope cells with the P38MAPK inhibitor, SB203580, augmented rather than prevented induction of apoptosis in the presence of increased GADD45 β . Together, these data suggest a novel role for p38 as a pro-survival pathway in pituitary tumors, and a novel mechanism of apoptosis induction by GADD45 β . Given its uniform loss and its many roles in tumorigenesis, GADD45 β represents a promising target for further mechanistic studies and a possible therapeutic target.

Sources of Research Support: VA Merit Review to MEW UCD Cancer Center and Genomics Core.

Nothing to Disclose: KAM, AJK, MX, KK-V, MGE, BKK-D, KOL, MG, MEW

Pub # P1-264

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Pro-Survival and Tumorigenicity of LAG1 Homolog Ceramide Synthase 6 (LASS6) in Gonadotrope Pituitary Tumors

Author String K Kiseljak-Vassiliades, M Xu, AJ Knox, MG Edwards, KA Michaelis, BK Kleinschmidt-DeMasters, KO Lillehei, M Geraci, ME Wierman
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Body Despite the relatively common frequency of gonadotrope tumors and concomitant morbidity, factors contributing to their onset or progression are poorly understood. Using DNA expression microarrays, bioinformatic tools and systematic literature review, LASS6 was identified as a novel candidate with significant overexpression (6.6- fold) in gonadotrope tumors (N=14) compared to normal pituitaries (N=9). Comparison of aggressive (N=7, >2cm and invasion) and nonaggressive (N=7, <2cm, no invasion) tumors showed that LASS 6 was significantly higher in the aggressive tumors (1.7- fold), implicating LASS6 as a potential prognostic marker. LASS6 is one of six ceramide synthase enzymes responsible for *de novo* ceramide synthesis with different fatty acid lengths incorporated into cellular membranes to modulate events such as apoptosis and differentiation. Although initially studies suggested that ceramides are uniformly pro-apoptotic, recent work suggests that LASS1-6 play distinct tissue specific roles in normal physiology and in tumor growth. LASS6 mRNA levels are elevated in breast and head and neck cancer cells, while suppressed in lung cancer. We asked the cell specificity of upregulation of LASS6 in human pituitary tumors. LASS6 mRNA and protein were increased in gonadotrope and ACTH-cell tumors, and low or undetectable in prolactinomas and growth hormone tumors, as well as in normal pituitaries. To assess its functional role in pituitary cells, LASS6 was transiently overexpressed (4.2-fold) in α T3 mouse gonadotrope cells. Increases in LASS6 protected cells from serum withdrawal induced apoptosis at 48h as assessed by caspase3 cleavage (2.2-fold). Pituitary cells stably overexpressing LASS6 were plated on soft agar to examine the role of LASS6 in tumorigenicity. After 22 days, LASS6-stable cells formed increased number of colonies compared to vector controls (26.3 \pm 2.9, 14.3 \pm 2.3, respectively P<0.03), a 1.9- fold increase in colony formation. In summary, LASS6 is upregulated in gonadotrope and ACTH tumors compared to normal pituitary. Overexpression of LASS6 in pituitary gonadotrope cells promotes survival and colony formation in soft agar, positioning LASS6 as a promising candidate in pituitary tumorigenesis and a potential prognostic and therapeutic target.

Sources of Research Support: VA Merit Review to MEW, UCD Cancer Center and Genomics Core.

Nothing to Disclose: KK-V, MX, AJK, MGE, KAM, BKK-D, KOL, MG, MEW

Pub # P1-265

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Whole Exome Sequencing for the Identification of the Gene(s) Causative for Pituitary Somatotrophic Hyperplasia in a Family with Gigantism and Acromegaly

Author String MF Azevedo, A Horvath, C Wassif, M Keil, P Hofman, I Holdaway, S Butler, J Niemela, JB Oliveira, E Oldfield, F Porter, CA Stratakis
NIH, Bethesda, MD; NIH, Bethesda, MD; NIH, Bethesda, MD; NIH, Bethesda, MD; University of Auckland, Auckland, New Zealand; Greenlane Clinical Centre, Auckland, New Zealand

Body Acromegaly and gigantism are caused by excessive growth hormone (GH) secretion. GH-secreting pituitary adenomas are the cause of the disease in more than 90% of cases, whereas pituitary hyperplasia is described in patients harboring hypothalamic or peripheral neuroendocrine tumors secreting GH-releasing hormone (GHRH). We studied a family that presented with gigantism, in which GH hypersecretion was due to pituitary hyperplasia with no evidence of ectopic GHRH secretion. In order to search for genes possibly involved in the pathogenesis of this Mendelian disease, we sequenced the whole exome of all four family members. The proband presented with accelerated growth, coarse facial features, enlarged hands and feet at the age of 2. GH IGF-I and prolactin levels were high and an enlarged pituitary was evidenced on MRI. Hypophysectomy was performed at ages 3 and 4, and the specimens were histologically described as mammosomatotroph hyperplasia. Similarly, his younger brother also presented with GH hypersecretion due to pituitary hyperplasia at age 2, and his mother was submitted to transsphenoidal surgery for the treatment of GH/prolactin hypersecretion early in her life (at age 3). We did not find any mutations in *MEN1*, *PRKARIA*, *GNAS*, *AIP*, *GHRH* and *GHRHR* genes. Whole exome sequencing showed 914 novel mutations in the germline DNA sample from the unaffected father (including missense, nonsense and splice site variations), 1124 in the mother, 2053 in the proband and 1697 in his brother. Data were filtered through removal of all mutations also found in the whole exome sequencings from 4 unrelated individuals who did not present the phenotype (negative controls). 40 mutations were present in the three affected individuals and absent in the unaffected father, all of them were missense substitutions. Pathway analysis showed that some of these genes are related to cell proliferation and tumorigenesis in various tissues. Sanger sequencing to confirm the presence and segregation of these mutations, as well as *in vitro* studies, are ongoing. Although intensively studied, the pathogenesis of most pituitary diseases remains elusive. Exome sequencing is a promising strategy to discover genes responsible for rare diseases, and in this case, to identify additional genetic causes of familial GH excess leading to gigantism.

Nothing to Disclose: MFA, AH, CW, MK, PH, IH, SB, JN, JBO, EO, FP, CAS

Pub # P1-266

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Mutation Analyses of PROP1 in 24 Patients with Combined Pituitary Hormone Deficiency: Identification of a Novel Substitution

Author String DC Moraes, KSSC dos Reis, GD Lorenzoni, FL Conceicao, M Vaismann, TM Ortiga Carvalho
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Hospital Universitario Clementino Fraga Filho - Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Body Introduction: Combined Pituitary Hormone Deficiency (CPHD) is defined as the presence of hormone deficits affecting at least two anterior pituitary lineages. The genetic form of CPHD may be originated from mutations in pituitary transcription factors (PTF) and the phenotypic profile of each case can give a clue of which PTF is most probably mutated (2, 4).
Patients and Methods: Twenty-four Brazilian patients with different manifestations of CPHD were examined for mutations affecting POU1F1, PROP1 and HESX1, three PTF genes. Genomic DNA was isolated from peripheral blood from these patients using a commercial kit (Wizard Genomic DNA Purification - A1120 - Promega). All exons of POU1F1, PROP1 and HESX1 were amplified by direct-PCR using intronic primers previously described (3). PCR products were treated with QIAquick PCR Purification Kit (Qiagen). The products were then directly sequenced using an ABI 3100 automatic DNA sequencer.
Results and Conclusion: No mutation or polymorphism was found in the POU1F1 or HESX1 genes of all patients studied. Seventeen percent of the patients present a frequent polymorphism already described in the literature, in the first exon of PROP1, a T>C substitution in nt27, with no change of amino acid (3). We also identified in two patients blood-related the most frequent mutation already described in PROP1 gene, the deletion AG 301-302, resulting in a truncated protein (1, 5). Our most important result was the identification of a novel homozygous substitution of a single base pair (nt 424) in PROP1, exon3, resulting in a change of an amino acid, in one patient with CPHD (GH, TSH and ACTH deficiencies), normal pituitary and absence of the septum pellucidum shown in Magnetic Resonance Imaging. For describe more specifically this last result we are submitting the mutation to functional tests.

- (1) Cogan J.D et al. J Clin Endocrinol Metab 1998; 83(9):3346-3349.
- (2) Cohen LE and Radovick S. Endocr Rev 2002; 23(4):431-442.
- (3) Deladoey J et al. J Clin Endocrinol Metab 1999; 84(5):1645-1650
- (4) Kelberman D and Dattani MT. Hormone Research 2007; 68(5):138-144
- (5) Wu W et al. Nat Genet 1998; 18(2):147-149.

Nothing to Disclose: DCM, KSSdR, GDL, FLC, MV, TMOG

Pub # P1-267

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Processing of Proaugurin Is Required to Suppress Proliferation of Tumor Cell Lines

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Body Augurin is a secretory molecule produced in the pituitary, thyroid and esophagus and is implicated in a wide array of physiological processes, from ACTH release to tumor suppression. However, the specific proaugurin-derived peptides present in various cell types are not yet known. In order to shed light on the posttranslational modifications required for biologic activity, we here describe the posttranslational processing of proaugurin in AtT-20 and Lovo cells and identify proaugurin-derived products generated by convertases. In vitro cleavage of proaugurin with proprotein convertases produced multiple peptides, including a major product with a mass of 9.7 kDa identified by mass spectrometry. Metabolic labeling of C-terminally tagged proaugurin in AtT-20 and AtT-20/PC2 cells resulted in a major 15 kDa tagged form on SDS-PAGE, which likely corresponds to the 9.7 kDa in vitro fragment, with the added tag, its linker and posttranslational modification(s). The secretion of neither proaugurin nor this cleavage product was stimulated by forskolin, indicating its lack of storage in regulated secretory granules and lack of cleavage by PC2. Incubation of cells with the furin inhibitor nona-D-arginine resulted in impaired cleavage of proaugurin, while metalloprotease inhibitors did not affect proaugurin proteolysis. These data support the idea that proaugurin is cleaved by furin and secreted via the constitutive secretory pathway. Interestingly, proaugurin was sulfated during trafficking through the secretory pathway and sulfation was completely inhibited by brefeldin A. Proliferation assays with three different tumor cell lines demonstrated that only furin-cleaved proaugurin could quell cell proliferation, suggesting that proteolytic cleavage of proaugurin is a posttranslational requirement to suppress cell proliferation.

Sources of Research Support: NIH grant R21 DK08448102 to IL.

Nothing to Disclose: AO, AL, IL

Pub # P1-268

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Stimulatory Role of Neurokinin B in the Central Control of Female Puberty and Its Modulation by Metabolic Status

Author String VM Navarro, F Ruiz-Pino, D Garcia-Galiano, MA Sanchez-Garrido, SJ Hobbs, M Manfredi, S Leon, JM Castellano, DK Clifton, L Pinilla, RA Steiner, M Tena-Sempere
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Body Studies of human genetics have revealed that *neurokinin-B (NKB)* and its receptor, *NK3R*, are essential regulators of reproduction. In rodents, sheep, goats and primates, *NKB* (the homologue of the human *TAC3* gene) is expressed in *Kiss1* neurons within the arcuate nucleus (ARC). Those neurons are thought to drive gonadotropin-releasing hormone (GnRH) and LH secretion, suggesting that NKB signaling plays a key role in the regulation of the neuroendocrine reproductive axis. To investigate the functional role of NKB-GnRH signaling in puberty, we mapped the distribution of *NKB* and *NK3R* in the brain of peri-pubertal (PND 35) female rats and demonstrated that *NKB* and *NK3R* mRNAs are broadly expressed throughout the brain, with prominent expression in the lateral hypothalamic area (LHA) and the medial basal hypothalamus. The expression of *NKB* remained unaltered across the transition between the late infantile (PND 20) and early pubertal (PND 35) periods; in contrast, the expression of *NK3R* tended to increase ($p = 0.07$) in the ARC across the pubertal transition. Central administration of senktide, a selective agonist of NK3R, elicited a brisk and robust increase in serum levels of LH in pre-pubertal animals (PND 25); moreover, a chronic infusion of SB-222200, an NK3R antagonist, at puberty evoked a delay in the timing of vaginal opening (VO) and modestly decreased levels of LH. The expression of *NKB* and *NK3R* in the hypothalamus was altered as a function of metabolic status, reflected by a marked decrease in the expression of *NK3R* mRNA (and to a lesser extent, *NKB* mRNA) in the ARC following a 48-h fast. Curiously, the acute LH response to senktide in pubertal females was augmented under fasting conditions, suggesting a sensitization of the NKB-NK3R-GnRH signaling pathway with metabolic stress. Moreover, repeated administration of senktide to pubertal female rats maintained under chronic subnutrition (a 30% reduction in daily caloric intake between PND 23 and 37) rescued VO (in 75%) and evoked a robust LH secretory response. These observations provide evidence for an inductive role of NKB-NK3R-GnRH signaling in the initiation of puberty and suggest that alterations in NKB signaling contribute to pubertal disorders linked to stress and negative energy balance.

Sources of Research Support: Eunice Kennedy Shriver NICHD/NIH through cooperative agreements U54 HD12629 (to the University of Washington Center for Research in Reproduction and Contraception), NICHD/NIH Grant R01 HD27142, NIDA/NIH RO1 DA016898 & KO5 DA020570; research grants BFU 2008-00984 (Ministerio de Ciencia e Innovación, Spain) and P08-CVI-00603 (Junta de Andalucía, Spain), and the Marie Curie OIF from the 7th Framework Programme of the European Union.

Nothing to Disclose: VMN, FR-P, DG-G, MAS-G, SJH, MM, SL, JMC, DKC, LP, RAS, MT-S

Pub # P1-269

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Disruption of Zebrafish Prokineticin 2 Signaling Causes Kallmann Syndrome-Related Phenotypes

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Body Endocrine gonadotrophin-releasing hormone (GnRH) cells play a crucial role in the establishment of the hypothalamic-pituitary-gonadal (HPG) axis in many species. Mutations in a variety of genes results in Kallmann Syndrome (KS) characterized by anosmia (loss of sense of smell) and hypogonadotrophic hypogonadism (HH) which is caused by the lack of endocrine GnRH cells from the hypothalamus. Recent studies have found that the prokineticin2 (pk2) and prokineticin receptor2 (pkr2) genes are involved in the development of olfactory bulb and reproductive system and mutations in either of these genes result in a phenotype similar to KS. Using zebrafish as a model system we are investigating the role of the pk2 and pkr2 genes in the development of hypothalamic, pituitary and olfactory tissues.

Using the mouse pk2 and pkr2 genes, we identified genes in zebrafish coding for predicted proteins having portions of high identity with pk2 and pkr2 genes. We found two homologues of the mouse pkr2 gene, pkr2a and pkr2b, having 69% and 62% identity at the amino acid respectively. We found one homologue of pk2 mouse gene with a 65% identity at the amino acid level. RT-PCR analysis showed that these genes are expressed in zebrafish larvae and adult brain tissues. Using antisense digoxigenin labelled specific probes we show that the pk2 gene is expressed in the adult hypothalamus. We used morpholinos (MO) to knockdown pkr2a, pkr2b and pk2 gene function, and examined the development of hypothalamic, pituitary and olfactory tissues using specific markers. Knockdown of pkr2a, pkr2b and pk2 proteins caused a significant reduction in the number of endocrine GnRH and oxytocin-like cells (both hypothalamic cell types) compared with controls. In addition, the anterior pituitary was malformed, as assayed by POMC-GFP cell lineages, in the MO treated animals compared to controls. Finally, we found that pk2, pkr2a and pkr2b MO treated embryos showed abnormal olfactory sensory axonal projections in the olfactory bulbs. Correlated with this defect was lack of emx1 expression in the developing OB.

In summary, we have shown that zebrafish have homologues of the mouse pk2 and pkr2 genes, with an apparent duplication of pkr2. Knockdown of pk2, pkr2a and pkr2b gene function results in abnormal development of the olfactory system and hypothalamic cell types, a phenotype resembling the clinical manifestations of KS.

Sources of Research Support: FONDECYT 1071071 and NIH/HD050820 Grants, MIDEPLAN Millenium Scientific Initiative Program, MECESUP and CONICYT scholarships.

Nothing to Disclose: JL, KEW

Pub # P1-270

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Accurate Detection of Intragenic Deletions of KAL1 Gene Using the Multiplex Ligation-Dependent Probe Amplification in Patients with Kallmann Syndrome

Author String LR Montenegro, MG Teles, L Gontijo, C Tusset, AP Abreu, M Castro, E Costa, A Lofrano-Porto, MT Batista H Garmes, AC Latronico, BB Mendonca, E Trarbach
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Body Introduction: Kallmann syndrome is a developmental disorder defined by the association of isolated hypogonadism hypogonadotropic with anosmia or hyposmia. Loss of function mutations in the KAL1 gene, mapped to Xp22.3, is responsible for the X-linked form of Kallmann syndrome and has been detected in 40-50% of these patients. The KAL1 gene spans 14 exons and has a highly homologous pseudogene (KALP) on Yq11. Objective: To detect KAL1 gene defects using PCR followed by DNA sequencing and multiplex ligation-dependent probe amplification (MLPA) in patients with Kallmann syndrome. Patients and Methods: 104 patients with Kallmann syndrome (91 males) were selected. Genomic DNA was extracted from peripheral blood and the entire coding region of the KAL1 gene were amplified and sequenced. In addition, gene dosage analysis was carried out using the SALSA MLPA kit P132 Kallmann-1 designed to detect KAL1 exonic deletion. Results and Discussion: Overall, we detected thirteen (12.5%) abnormalities in the KAL1 gene: five stop codon mutations (38.4%), two frameshift (15.4%), one intronic mutation (7.6%), and five intragenic deletions (38.4%). Intragenic deletions detected by PCR in two consecutive patients, appeared to encompass exons 5-10 and exon 1 of KAL1, respectively. However, in MLPA the deletions encompass exons 5-14 and exons 1-2, respectively. Sequencing of PCR products of exons 11-14 and 2 revealed that these amplicons were originated from KALP, suggesting that Yq11 sequence could be amplified by PCR in absence of KAL1. Conclusions: These results indicate that intragenic exon deletions are one the most frequent abnormalities of KAL1 gene and its detection is more precise and accurate using MLPA. This technique could be considered a simpler and faster initial screening method for the molecular diagnosis of KAL1 mutations in patients with X-linked Kallmann syndrome.

Sources of Research Support: FAPESP 05/04726-0.

Nothing to Disclose: LRM, MGT, LG, CT, APA, MC, EC, AL-P, MTB, HG, ACL, BBM, ET

Pub # P1-271

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Cell-Type-Specific Modulation of PC2-Mediated Precursor Processing by 7B2

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Body The neuroendocrine protein 7B2 is required for the production of active prohormone convertase 2 (PC2), an enzyme involved in the maturation of peptide hormones within neuronal and endocrine tissues. Whether 7B2 levels can dynamically modulate peptide production through regulation of PC2 activity remains unclear. We have here investigated the relationship between 7B2 levels and PC2 activity and precursor processing in four cell systems: primary pituitary cultures obtained from 7B2 null mice; AtT-20/PC2 cells (AtT-20 cells stably expressing PC2); RinPE cells (Rin5f cells stably expressing proenkephalin); and α -TC-6 cells. When primary pituitary cultures prepared from 7B2 null mice were infected with 7B2-encoding adenovirus, increased α -MSH and reduced ACTH content was observed, supporting the idea that 7B2 expression mediates PC2-mediated POMC processing. In contrast, infection of AtT-20/PC2 cells with 7B2-encoding adenovirus resulted in greatly increased secreted PC2 activity, but affected neither processing nor secretion of cellular POMC-derived products. siRNA-mediated knock-down of 7B2 in these cells also effected no change in α -MSH content. A similar result was observed in RinPE cells, i.e., 7B2-encoding adenoviral infection resulted in increased secretion of PC2 activity but did not increase cellular PC2-mediated precursor processing. In contrast, infection of the pancreatic alpha cell line α -TC6 with 7B2-encoding adenovirus efficiently increased production of immunoreactive glucagon, while siRNA-mediated knock-down of 7B2 resulted in significantly decreased cellular content of this peptide. In contrast to AtT-20 and RinPE cells, in α -TC6 cells 7B2 overexpression in resulted decreased secretion of active PC2. These results indicate that 7B2 modulation of PC2-mediated peptide production is cell-specific, occurring in primary cultures of pituitary tissues from 7B2 null mice and in α -TC6 cells, but not in AtT-20/PC2 or Rin cells. We hypothesize that 7B2 may act to route proPC2 to secretory granules in α -TC6 cells, but not in AtT-20/PC2 or RinPE cells, thus achieving cell-type specific precursor processing efficacy.

Sources of Research Support: NIH Grant DK49703 to IL. M.H. is grateful to the German Academy of Sciences Leopoldina Foundation (LPDS 2009-33) for postdoctoral funding.

Nothing to Disclose: MH, S-NL, JRH, IL

Pub # P1-272

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title On the Effect of CRH and Dexamethasone on POMC Synthesis and ACTH Secretion in Rat Pituitary Primary Cultures

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Body *Pomc* synthesis and ACTH secretion by the anterior pituitary are controlled by two major, opposing players namely CRH and glucocorticoids. To date, most studies on ACTH synthesis and secretion have been performed on the AtT-20 mouse corticotroph tumor cell line or on *Pomc*-transfected cells. Aim of the present study was to evaluate the effect of CRH and DEX on *Pomc* expression and ACTH secretion in normal rat corticotropes *in vitro*. Methods. Rat anterior pituitary primary cultures were incubated with 10 nM CRH or 10 nM DEX for 4h, 24h, 48h, 72h, 96h and 120h prior to medium and RNA collection. *Pomc* gene expression was evaluated by real time PCR and ACTH was measured by IRMA. Results: CRH stimulated ACTH secretion at 4h (control 0.5±0.03 ng/ml, CRH: 2.9±0.25 ng/ml, p<0.05) and the effect was maintained up to 120h (control 2.2±0.68 ng/ml, CRH: 8.3±0.49 ng/ml at 24h; control 4.2±0.28 ng/ml, CRH: 14.0±0.96 ng/ml at 48h; control 5.4±0.54 ng/ml, CRH: 21.7±1.04 ng/ml at 72h; control 7.0±0.44 ng/ml, CRH: 26.9±2.23 ng/ml at 96h, control 8.4±0.52 ng/ml, CRH: 32.8±2.76 ng/ml at 120h, all p<0.05); stimulation of *Pomc* synthesis was evident after 24h (1.3±0.1 vs *Hprt*, p<0.05) and increased progressively over time (1.3±0.1 at 48h, 1.6±0.1 at 72h, 2.0±0.1 at 96h and 2.8±0.3 at 120h vs *Hprt* all p<0.05). DEX clearly inhibited ACTH secretion starting at 4h (0.4±0.03 ng/ml at 4h, 1.3±0.71 ng/ml at 24h, 2.1±0.16 ng/ml at 48h, 2.4±0.34 ng/ml at 72h, 3.2±0.40 ng/ml at 96 h and 2.9±0.26 ng/ml at 120h; all p<0.05), but had no effect on *Pomc* gene expression at any time point (1.2±0.1 at 4h, 1.1±0.1 at 24h, 1.1±0.1 at 48h, 1.1±0.1 at 72h, 1.3±0.1 at 96h and 1.1±0.1 at 120h vs *Hprt*, all N.S.). Conclusions: The present study shows that CRH stimulates ACTH synthesis and secretion by normal corticotropes while dexamethasone blunts ACTH release but appears to have no effect on *Pomc* synthesis. This data contrasts with results obtained in tumoral corticotropes or transfected cells and points to the need for studies on normal corticotropes in order to better understand HPA axis physiology.

Nothing to Disclose: FPG, MFC, LP, FC

Pub # P1-273

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Effects of Retinoid X Receptor on ACTH Secretion and POMC Gene Expression in AtT20 Corticotroph Cells

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Body

Introduction: Retinoic acid suppresses adrenocorticotrophic hormone (ACTH) secretion in ACTH-dependent Cushing's syndrome, and is a candidate agent for its drug treatment. However, the role of retinoid X receptor (RXR) in ACTH secretion and proopiomelanocortin (POMC) gene expression has not been clarified. We therefore examined the effects of RXR on ACTH secretion and POMC gene regulation in ACTH secreting AtT20 cells.

Methods: Mouse pituitary corticotroph AtT20 cells were transfected with POMC promoter reporter vectors, and their luciferase activity was thereafter measured. POMC mRNA expression level in AtT20 cells was determined by real-time PCR methods. ACTH secretion from AtT20 cells to the media was measured by ELISA. AtT20 cell number was determined using WST-8.

Results: A synthetic RXR agonist HX630 suppressed ACTH secretion and POMC mRNA expression in AtT20 cells at 10 [μ]mol/L. An overexpression of RXR α in AtT20 cells decreased POMC gene promoter activity, and HX630 augmented the RXR α -mediated its suppression. An overexpression of a constitutively active RXR α mutant suppressed the promoter activity stronger than that of wild type RXR α . HX630 also decreased AtT20 cell number.

Conclusion: The RXR agonist HX630 and the overexpression of RXR decreased the POMC gene expression in AtT20 cells. RXR may thus be a target of the retinoic acid-mediated POMC gene suppression.

Nothing to Disclose: AU, KM, MK, YI, SI, AS

Pub # P1-274

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Cytotoxic and Apoptotic Effect of Gossypol on Mouse Pituitary Corticotroph Tumor AtT20/D16v-F2 Cells: Could It Be a Novel Therapeutic Agent for Cushing Disease?

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Body
Objective: Cushing disease results from excess adrenocorticotrophic hormone secreted by pituitary corticotroph tumors. Standard treatment including surgery and radiotherapy is not successful all the time and recurrence is frequent. Gossypol (AT-101), which is a polyphenolic compound extracted from cotton plant (*Gossypium species*), inhibits the proliferation of several tumor cell lines; however, its effect on pituitary tumor cell proliferation has not been determined. Our objective was to determine whether gossypol inhibits proliferation of corticotroph cell line.
Material-Methods: Mouse corticotroph AtT20/D16v-F2 cells was treated with increasing concentrations of AT-101 (-/- gossypol, 0.5-30 [micro]M) for 24-48-72-96-120 hours. Cell viability was determined by XTT Cell Proliferation Assay (Roche). Apoptosis was detected by Cell Death Detection Elisa Plus Kit (Roche) and confirmed by Caspase-Glo 3/7 Assay.
Results: AT-101 inhibited cell proliferation of AtT-20 corticotroph cells in a time and dose dependent manne and induced apoptosis in AtT20 corticotroph cells.
Conclusion: We demonastrate that gossypol inhibits pituitary tumor cell proliferation and induces apoptosis. We propose that gossypol should be further evaluated as a novel therapeutic agent in management of Cushing disease.

Nothing to Disclose: BY, AK, HA, BK, RU, SC, GO, CY, FS

Pub # P1-275
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)
Title Poly(A) Tail Length of Vasopressin Is Shortened in a Mouse Model of Familial Neurohypophysial Diabetes Insipidus
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Body Familial neurohypophysial diabetes insipidus (FNDI), an autosomal dominant disorder, is mostly caused by mutations in the gene encoding neurophysin II (NPII), the carrier protein of arginine vasopressin (AVP). We generated a mouse model for FNDI possessing a heterozygous point mutation (Cys98stop) in the NPII gene. The FNDI mice manifested progressive polyuria, aggregate formation in the endoplasmic reticulum (ER) of AVP cells, and reduction of AVP mRNA expression in the hypothalamus (1,2,3). In this study, we examined the mechanisms by which AVP mRNA expression is reduced in FNDI mice. The expression levels of AVP mRNA were significantly lower in FNDI mice compared to wild-type mice in basal condition as well as after hypertonic saline injection or water deprivation. On the other hand, the expression levels of AVP heteronuclear (hn) RNA, a sensitive indicator for gene transcription, were similar between FNDI and wild-type mice in both basal and osmotically stimulated conditions. The analyses of cDNA demonstrated that expression of mutant AVP mRNA in the hypothalamus was similar to that of normal AVP mRNA in FNDI mice. These data indicate that expression of both normal and mutant AVP mRNA was decreased posttranscriptionally in FNDI mice. Northern blot analyses demonstrated that decreases in AVP mRNA expression were accompanied by shortening of its poly(A) tail length in FNDI mice. While the expression levels or poly(A) tail length of oxytocin (OT) were not different between FNDI and wild-type mice, incubation of hypothalamic explants of wild-type mice with thapsigargin or tunicamycin, both of which are known as ER stressors, caused shortening of poly(A) tail length of both AVP and OT mRNAs, accompanied by decreases in their expression. On the other hand, tauroursodeoxycholate, a molecular chaperone reducing ER stress, increased the size of poly(A) tail of AVP and OT mRNA, accompanied by increases in their expression in the hypothalamic cultures. Thus, our data provide a novel mechanism that ER stress decreases poly(A) tail length as well as mRNA expression of neurohypophysial hormones, probably to reduce the load of unfolded proteins.

- (1) Arima et al., *J Neuroendocrinol* 2010; 22, 754
- (2) Hiroi, Morishita et al., *Am J Physiol Regul Integr Comp Physiol* 2010; 298, R486
- (3) Hayashi et al., *Am J Physiol Regul Integr Comp Physiol* 2009; 296, R1641

Nothing to Disclose: YM, HA, MH, MH, DH, NA, NO, YS, HN, YO

Pub # P1-276

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Nitric Oxide Reduces Hemorrhage-Induced Vasopressin and Oxytocin Neuron Activity and mRNA Expression in Hypothalamic Paraventricular and Supraoptic Nuclei

Author String WL Reis, RC Rorato, LF Bastianini, LL Elias, J Antunes-Rodrigues
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Body Several studies have demonstrated that hypovolemia induces an increase in neuronal nitric oxide synthase (nNOS) activity in hypothalamic nuclei, suggesting that nitric oxide (NO) is involved in the regulation of vasopressin (VP) and oxytocin (OT) secretion. We have shown that central NO donor reduces plasma VP and OT concentrations in response to hemorrhage (HEM). In this study, we examined the role of central nitricergic system in the VP, OT and nNOS neuron activity and their mRNA gene expression in the paraventricular (PVN) and supraoptic (SON) nuclei of rats submitted to acute HEM. Wistar male rats (280g, 5/group) were anaesthetized with 2.5% tribromoethanol (1ml/100g, ip) for intracerebroventricular cannulae placement. Rats were centrally pretreated with vehicle (0.15M NaCl), NOS inhibitor (L-NAME 250[micro]g) or NO donor (SNAP 5[micro]g), and 20min after, they were submitted to intra-arterial hypovolemia (15% of total blood volume, 1ml/100g/min). PVN and SON nuclei were collected by decapitation 60min after HEM for mRNA expression using Real Time PCR or, by perfusion 90min after HEM for neuronal activation using fluorescent c-Fos immunohistochemistry. The results are reported as means±SEM and the data were analyzed by Two-Way analysis of variance followed by Newman-Keuls post-hoc test. In the PVN and SON, HEM increased VP (3.2±0.3au), OT (3.3±0.4au) and nNOS (2.8±0.3 arbitrary units, au) mRNA expression. Pretreatment with L-NAME did not change HEM-induced VP and OT expressions, but it reduced nNOS mRNA expression (1.1±0.2au). In contrast, SNAP decreased the VP (1.1±0.2au) and OT (1.2±0.2au) mRNA expression induced by HEM, without affecting nNOS expression in both structures. Activation of VP, OT and nNOS neurons in both PVN and SON were increased (32.4±5% VP, 23.1±3% OT, 29.5±3% nNOS) by HEM. Pretreatment with L-NAME reduced VP (28.2±4%) and nNOS (14.5±1%) neuron activity, but it did not affect OT, in the PVN and SON nuclei after HEM. On the other hand, SNAP decreased the VP (27.3±3%) and OT (17.6±2%) neuron activity, with no changes in the nNOS, in the PVN and SON nuclei induced by HEM. The present data indicate that HEM increase VP, OT and nNOS mRNA expression and neuronal activity and, exogenous NO reduces these effects in response to HEM, suggesting an inhibitory modulation of NO in the control of neurohypophysial hormone synthesis and activity during hypovolemia.

Sources of Research Support: FAPESP and CNPq; Technical Assistance: Maria Valci A dos Santos and Milene M Lopes; The experimental procedures were approved by the Ethical Committee for Animal Use of the School of Medicine of Ribeirao Preto (n[ordm] 029/2006).

Nothing to Disclose: WLR, RCR, LFB, LLE, JA-R

Pub # P1-277

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Arginine Vasopressin Immunoreactivity in the Suprachiasmatic Nucleus Is Decreased in Patients Treated for : Suprasellar Tumor Leading to Visual Field Defects

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Body

Background
Suprasellar tumors with compression of the optic chiasm are associated with an impaired sleep-wake rhythm. We hypothesized that this reflects a disorder of the biological clock of the human brain, the suprachiasmatic nucleus (SCN), which is located just above the optic chiasm. In order to test this hypothesis, we investigated whether two key neuropeptides of the SCN, i.e. arginine vasopressin (AVP) and vasoactive intestinal peptide (VIP), were expressed to a lower extent in patients treated for a suprasellar tumor inducing permanent visual field defects.

Methods
Hypothalami of five such patients (acromegaly n=2, nonfunctioning macro-adenoma n=1, macroprolactinoma n=1, infundibular metastasis of a colorectal adenocarcinoma n=1) and of sixteen age- and gender-matched controls were obtained from the Netherlands Brain Bank. AVP- and VIP-immunoreactivity was quantified using each 50th 6[μm] coronal section throughout the SCN followed by optic density measurements. Total immunoreactivity was estimated according to Cavalierre's principle.

Results
Total AVP-immunoreactivity in the SCN was lower in patients with a suprasellar tumor than in controls (one-sided Mann Whitney U test, $p = 0.046$). By contrast, total VIP-immunoreactivity was not different between patients and controls ($p = 0.274$).

Conclusion
Suprasellar tumors leading to permanent visual field defects are associated with reduced AVP-, but not VIP-immunoreactivity in the SCN. These findings raise the possibility that selective damage to the SCN may contribute to sleep-wake disturbances in these patients.

Nothing to Disclose: AJB, EF, JES-W, DFS, PHB, AA

Pub # P1-278

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title The Effects of Ablation of Vasopressin and Oxytocin Neurons on Social Behaviors in Mice

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Body The biochemical structure and expression patterns of the neuropeptides, Arginine Vasopressin (AVP) and oxytocin (OT) are highly conserved during evolution in species ranging from fish to primates. When released from the posterior pituitary, both produce well-known peripheral effects: AVP regulates vasoconstriction and renal water resorption; OT facilitates uterine contractions and milk ejection. In addition, AVP and OT also act as neuromodulators regulating various social behaviors, although the specific behaviors that they regulate are species dependent.

We used a virus-assisted conditional ablation strategy to investigate the function of AVP and OT regulating social behaviors in mice. We have generated AVP::Cre and OT::Cre transgenic mice lines in which these neuropeptide-expressing neurons are labeled with the Cre recombinase. In order to achieve temporally and spatially controlled ablation of neurons, a recombinant adeno-associated virus (AAV) line was constructed to conditionally express the human Diphtheria toxin receptor (DTR) after Cre-mediated recombination. Since mice and rats are insensitive to Diphtheria toxin due to the lack of endogenous receptors, only those Cre-expressing neurons that have been infected with AAV-DTR will be killed upon injection of Diphtheria toxin. We demonstrate that this strategy is highly effective to selectively kill AVP or OT expressing neurons in the adult. Further, we demonstrate that targeted ablation of either the AVP neurons or the OT neurons in the paraventricular nucleus leads to a pronounced decrease in intermale aggression. Our results thus indicate that the AVP/OT neurons in the paraventricular nucleus are important for normal aggressive behaviors of mouse.

Nothing to Disclose: SY, CD

Pub # P1-279

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Identification of Two Variants in *PROKR2* Gene in a Cohort of Patients with Congenital Hypopituitarism

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Body **Context:** Prokineticin receptor-2 (*PROKR2*), a G protein-coupled receptor, is essential for normal olfactory bulb development and sexual maturation. Recent studies showed that adenohipophyseal and olfactory placodes share a common embryological origin. There are previous reports that inactivating mutations in *PROKR2* gene could underlie some cases of congenital hypopituitarism, therefore, we tested this hypothesis in a Brazilian cohort. **Aim:** To investigate loss-of-function mutations in *PROKR2* gene in patients with combined pituitary hormone deficiencies (CPHD). **Patients and Methods:** Eighty-seven patients (54 males) with CPHD were studied. A group of 100 Brazilian individuals was used as controls. Genomic DNA was extracted from peripheral blood and the entire coding region of *PROKR2* gene was amplified and automatically sequenced. **Results:** Two distinct variants in heterozygous state, p.R85C and p.R248Q were identified in the *PROKR2* in two unrelated females born from non consanguineous parents. These variants were not found in the control group and both were previously reported in patients with congenital hypogonadotropic hypogonadism. Previous functional studies performed *in vitro* in these variants showed reduction of G α_q calcium mobilization and did not affect MAPK activation. No mutations in the coding region of *GLI2* and *LHX4* were found in both patients. We also ruled out mutations in the coding region of *HESX1* in the patient with *PROKR2* p.R248Q mutation. The p.R85C variant was identified in a female patient with GH, ACTH, LH and FSH deficiencies and partial diabetes *insipidus*. Neuroimaging revealed normal anterior pituitary, absent stalk and a non visualized posterior pituitary. Intriguingly, this patient reached normal final height without growth hormone treatment. The other variant p.R248Q was identified in another female with GH, TSH and partial ACTH and gonadotropin deficiencies. The MRI showed small anterior pituitary, absent stalk and undescended posterior pituitary. **Conclusion:** We identified two variants in *PROKR2* in two females with congenital hypopituitarism. The role of these *PROKR2* mutations and possibly their interaction with variants in yet unidentified partners or environmental factors in the pathogenesis of this condition remains to be established.

Sources of Research Support: Fundacao de Amparo a Pesquisa do Estado de Sao Paulo 05/04726-0.

Nothing to Disclose: FAC, CT, APA, ACL, LRC, VNB, IJPA, BBM

Pub # P1-280

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title A Window into the Brain: *In Vivo* Permeability of the Median Eminence and Functional Implications

Author String M Schaeffer, C Lafont, F Molino, F Langlet, E Trinquet, T Roux, L Nicolas, E Bourrier, G Osterstock, P-F Mery, P Verdie, J Marie, J Martinez, V Goffin, J-A Fehrentz, V Prevot, P Mollard
Institute of Functional Genomics, CNRS UMR5203, Montpellier, France; RCSI Research Institute, Dublin, Ireland; Jean-Pierre Aubert Research Center, INSERM U837, Lille, France; Parc Technologique Marcel Boiteux, Bagnols sur C[grave]ze, France; Institut des Biomolécules Max Mousseron, Faculty of Pharmacy, Universities of Montpellier 1 and 2, Montpellier, France; Research Center for Growth and Signaling, University Paris Descartes, Faculty of Medicine, Paris, France

Body The median eminence is located at the junction between the pituitary gland and the hypothalamus. Neuroendocrine neurons located in the arcuate nucleus of the hypothalamus secrete their inhibitory or stimulatory peptides at the level of fenestrated vessels of the median eminence. These neurohormones are carried to the pituitary where they act on target cells to generate hormone pulses involved in a range of physiological responses. In addition to neurons involved in endocrine function, neurons involved in the regulation of appetite are found in the arcuate nucleus. Blood-borne factors such as ghrelin, a hormone produced by the stomach and involved in both neuroendocrine function and food intake, could enter through the fenestrated capillaries of the median eminence and diffuse into the arcuate nucleus, providing either a retrograde control of neurohormone secretion and/or food intake. However, it is not clear how blood-borne factors can reach their target neurons in the brain, as diffusion of molecules from the median eminence to neuronal cells bodies is controlled by tanycytes, specialized ependymal cells that line the critical junctions to which may act to prevent circulating molecules from crossing the median eminence into the brain. To answer this question, we used state-of-the-art 2-photon microscopy and surgical approaches to measure diffusion dynamics of iv-injected fluorescent molecules in the median eminence, a structure which is located deep on the ventral side of the brain. We determined the cut-off size for vessels of the median eminence in vivo and investigated how far bioactive fluorescently-labeled ghrelin diffused into the arcuate nucleus. We found that this small 4 kDa molecule was able to diffuse beyond the tanycyte extensions and into the median eminence and arcuate nucleus. It specifically bound to and activated neurons in the area of diffusion, including those involved in the regulation of food intake, as measured using fluorescence intensity and c-Fos expression. These data provide an unprecedented insight into the role of the median eminence in molecule entry into the brain and provides a particularly good model for studying the peripheral control of hypothalamic function under different physiological stages or in pathological conditions such as deficits in pituitary hormone secretion or deregulations of energy balance and obesity.

Sources of Research Support: Grants from the Agence Nationale de la Recherche (ANR Pit-Net), Institut National de la Santé et de la Recherche Médicale (INSERM), Centre National de la Recherche Scientifique (CNRS), the Universities of Montpellier 1 and 2, National Biophotonics and Imaging Platform (Ireland) (NBIP), Fondation pour la Recherche Médicale (FRM), Réseau National des Génopoles, Institut Fédératif de Recherches No. 3, Région Languedoc Roussillon and the Medical Research Council (MRC).

Disclosures: ET: Researcher, Cisbio Bioassays. TR: Researcher, Cisbio Bioassays. LN: Employee, Cisbio Bioassays. EB: Employee, Cisbio Bioassays. Nothing to Disclose: MS, CL, FM, FL, GO, P-FM, PV, JM, JM, VG, J-AF, VP, PM

Pub # P1-281

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Possible Involvement of 16 kDa Prolactin (PRL) in the Pathophysiology of Hippocampal Neurons

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Body PRL is known to act as a neuropeptide of the brain such as anxiolysis, regulation of maternal behavior, and prevention of chronic stress-induced decrease of adult hippocampal neurogenesis. PRL is expressed in several brain regions including hypothalamus (neuronal PRL) and peripheral PRL also can access to central nervous system via crossing the blood brain barrier in the choroid plexus. 16kDa N-terminal fragment of PRL has been investigated in the field of vascular research as an active peptide to suppress angiogenesis, and cathepsin D is a candidate of the responsible proteolytic enzyme. However, the presence and roles of 16kDa PRL in the brain is not known. In this study, we examined the expression of PRL and PRL receptors in the rat hippocampal neurons and whether 16kDa PRL is generated in the brain.

[Material and methods] Organs including the brain, pituitary gland and liver were taken from Wistar female rats. Hippocampus was dissected under a stereomicroscope. mRNA expressions of PRL and PRL receptors were examined by real-time PCR. The protein expression of PRL receptors was also examined in cultured rat hippocampal neurons by immunofluorescence staining. Generation of 16kDa PRL was examined by incubating rat 23kDa PRL with a microsome fraction of the rat brain, and with cathepsin D as a positive control.

[Results] mRNA of PRL receptors was expressed in hippocampus and the relative amount was 2.04 ± 0.01 when the expression in the pituitary gland was 1. Immunofluorescence staining revealed that PRL receptors were present as a protein in rat hippocampal neurons. In contrast, PRL was not expressed in the hippocampus. Incubation of rat 23kDa PRL with the microsome fraction of rat brain at pH 7 produced 16 kDa PRL, while that with cathepsin D produced 16kDa PRL at pH 4. Although cleavage of 23kDa PRL to 16kDa PRL by cathepsin D was inhibited by pepstatin A, that by brain microsome fraction was not inhibited.

[Conclusion] PRL receptors were present in the hippocampus. The finding that 16kDa PRL was produced by co-incubation with rat brain microsome fraction at neutral pH and the non-inhibition by the blocker of cathepsin D (pepstatin A) suggests that unidentified enzyme other than cathepsin D might be involved in the generation of 16kDa PRL in the brain.

Nothing to Disclose: NH, AS

Pub # P1-282

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title A Novel Interaction of Bone Morphogenetic Protein and Growth Hormone-Releasing Peptide in Regulating Adrenocorticotropin Production by Corticotrope Cells

Author String N Tsukamoto, F Otsuka, T Miyoshi, M Takano, K Inagaki, E Nakamura, T Terasaka, T Ogura, Y Iwasaki, H Makino
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Body The mechanism by which GH releasing peptides (GHRPs) stimulate ACTH secretion by corticotropes has yet to be clarified. We studied the effects of GHRP-2 on ACTH secretion using mouse corticotrope AtT20 cells focusing on the biological activity of BMPs. GHRP-2 increased ACTH and cAMP synthesis in a concentration-responsive manner, in which GHRP-2 induction of ACTH production was less potent compared with the effects of CRH. BMP-4 exhibited a concentration-dependent suppression of basal ACTH production and transcriptional activities of POMC gene by AtT20 cells. The inhibition of BMP-Smad1/5/8 pathway led to increase in ACTH production by AtT20 cells, suggesting the inhibitory roles of endogenous BMPs on ACTH production. Of note, BMP-4 suppressed ACTH production induced by CRH more efficaciously than that induced by GHRP-2. In accordance with ACTH regulation, the reducing effects of BMP-4 on the POMC-promoter activity were prominent in the conditions induced by CRH compared with that induced by GHRP-2. BMP-4 had no significant effect on cAMP synthesis induced by CRH or GHRP-2. CRH stimulation readily activated MAPK and Akt phosphorylation in AtT20 cells, in which CRH-induced phosphorylation of ERK and p38 was suppressed by BMP-4. On the contrary, GHRP-2 had no effect on the activation of these pathways. CRH-induced ACTH production was significantly suppressed by inhibitors of ERK, p38 and Akt, whereas GHRP-2-induced ACTH levels were unaffected by these inhibitors. However, a cAMP-PKA inhibitor reversed CRH- as well as GHRP-2-induced ACTH secretion. Furthermore, the inhibition of ERK and p38 significantly reduced cAMP synthesis induced by CRH but not by GHRP-2. Thus, CRH activates ACTH production through ERK and p38 pathways in addition to cAMP-PKA pathway that is also activated downstream of MAPK. On the other hand, GHRP-2-induced ACTH production was predominantly linked to cAMP-PKA pathway. Moreover, CRH and GHRP-2 upregulated BMP receptor signaling, while BMP-4, CRH and GHRP-2 had no significant effect on the expression level of GH secretagog receptor. Taken it consider that cAMP as well as GHRP-2 suppressed the expression of Smad6/7, which is an inhibitor of BMP-Smad1/5/8, the induction of cAMP is likely to be linked to the upregulation of BMP receptor signaling. Collectively, a functional interaction between GHRP and BMP system is uncovered, in which endogenous BMP activity may act as an autoregulatory system in controlling cAMP-induced ACTH induction.

Nothing to Disclose: NT, FO, TM, MT, KI, EN, TT, TO, YI, HM

Pub # P1-283

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Genetic Variation in Bone Morphogenetic Protein 4 (*BMP4*) in Combined Pituitary Hormone Deficiency

Author String S Martens, J Breitfeld, D Schleinitz, K Dietrich, B Enigk, I Muller, R Pfaffle, J Klammt, M Stumvoll, P Kovacs, D Fuhrer, A Tonjes
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Body

Background & Aim: Mutations in transcription factors have been reported in rare cases of congenital hypopituitarism but the genetic causes for most patients with combined pituitary hormone deficiency remain unknown. The bone morphogenetic protein 4 (*BMP4*) is a member of the TGF- β superfamily and its gene maps on chromosome 14. As a developmental gene it plays a major role in eye, brain and digit development. Furthermore *BMP4* has been suggested to play a role in the maturation of the pituitary gland. Here, we investigated whether genetic alterations/variations/defects in *BMP4* could play a role in combined pituitary hormone deficiency.

Methods: Our study included 20 patients with combined pituitary hormone deficiency. MRI showed a hypoplastic or absent pituitary gland. Prior to analyses, carriers with mutations in *Prop*, *Pit1* and *HesX* have been excluded from the study. To screen for *BMP4* genetic variation all 4 exons (exons, exon-intron-boundaries, 5[acute]- and 3[acute]-UTRs) in all isoforms of the gene were directly sequenced. We used TaqMan technique for genotyping of identified novel variants in healthy controls. Further, we analyzed the conservation of single amino acid positions of *BMP4* by including sequences of 37 different species from publicly available databases.

Results: One patient harboured a heterozygous missense mutation in exon 4 of the coding region of *BMP4*. No carrier of the mutation was detected in a cohort of 1032 healthy subjects. The C/G substitution predicts an amino acid change from arginine to proline (p.R300P). Thereby, a dysfunction during development of pituitary gland caused by this mutation might be the reason for combined pituitary hormone deficiency in this patient. Comparative sequence analyses across 37 species revealed that this position is strongly conserved with exclusive existence of arginine in all species, which further supports the potential functional relevance of this mutation.

Discussion and Conclusion: This is the first report on a *BMP4* mutation in a patient with combined pituitary hormone deficiency. *BMP4* mutations have so far been suggested to account for developmental defects such as anophthalmia-microphthalmia, poly/syndactily and cleft palate. We suggest that the identified heterozygous missense mutation may explain combined pituitary hormone deficiency in this patient. Further functional analysis of *BMP4* in pituitary cells may provide novel insights in the pathogenesis of combined pituitary hormone deficiency.

Nothing to Disclose: SM, JB, DS, KD, BE, IM, RP, JK, MS, PK, DF, AT

Pub # P1-284

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Growth Hormone-Releasing Hormone (GHRH) Improves Post-Ischemic Left Ventricular Function and Decreases Ischemia/Reperfusion Injury in Rat Heart through Activation of the RISK and SAFE Pathways

Author String R Granata, C Penna, F Settanni, F Tullio, P Pagliaro, G Alloati, E Ghigo
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Body The hypothalamic neuropeptide GHRH has been recently shown to exert survival effects in isolated cardiomyocytes and to protect the rat heart from ischemia/reperfusion injury, given before ischemia (1). Since however, a treatment carried out after the ischemic insult (e.g. Postconditioning; PostC) is a more feasible practice for clinical application, we also studied the action of GHRH given during reperfusion on infarct size (IS), cardiac performance and activation of Reperfusion Ischemic Survival Kinases (RISK) and Survivor Activating Factor Enhancement (SAFE) pathways (2). Isolated rat hearts perfused at constant flow were subjected to: a) 30 min ischemia (I) and 120 min R (I/R), b) PostC (5 cycles of 10 s I/R at the beginning of R, c) GHRH (50 nM) within the first 20 min of R. We assessed left ventricular (LV) end-diastolic and LV developed pressures (LVDP) during R, IS and kinases phosphorylation (Western blotting) at the end of R. IS (61±4% of risk area in I/R) was significantly reduced in PostC and GHRH groups (28±3 and 17±2%, respectively). GHRH given in early R completely reversed the post-ischemic cardiac dysfunction. These effects were inhibited by the GHRH-receptor (GHRH-R) antagonist JV-1-36. GHRH promoted the phosphorylation of the RISK kinases, phosphoinositide 3-kinase (PI3K)/Akt and Glycogen Synthase Kinase-3β (GSK3β). Moreover, GHRH phosphorylated signal transducer and activator of transcription-3 (STAT-3), as part of the SAFE pathway, as well as eNOS and AMPK, whose activation has been shown to prevent myocardial injury and cardiomyocyte apoptosis. GHRH-induced activation of these pathways was reduced in hearts treated with JV-1-36. These results indicate that, like PostC, GHRH given during early reperfusion exerts cardioprotective effects, enhancing LVDP and reducing IS and development of diastolic contracture. Moreover, GHRH effects involve the GHRH-R and activation of RISK and SAFE pathways. Overall, these findings suggest therapeutic implications for GHRH and/or its agonists in heart failure and myocardial infarction.

1) Granata et al., *Cardiovasc Res*, 2009
2) Pagliaro P et al., *Antioxid Redox Signal* 2011; In press

Nothing to Disclose: RG, CP, FS, FT, PP, GA, EG

Pub # P1-285

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Reproduction & Neuroendocrinology (1:30 PM-3:30 PM)

Title Oral Delivery of Octreotide Acetate, a Synthetic Analog of Somatostatin, in Intravail[reg], Improves Uptake, Serum Half-Life, and Bioavailability over Subcutaneous Injection: A Pharmacokinetic Study in Male Swiss Webster Mice

Author String EE Maggio, P Grasso
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Body The most effective option for the medical treatment of patients with acromegaly is the use of somatostatin analogs. Octreotide acetate is a synthetic analog of somatostatin, with similar effects but a prolonged duration of action. Octreotide acetate is routinely given by subcutaneous (s.c.) or intramuscular injection. In the present study, we examined the feasibility of oral delivery of octreotide acetate reconstituted with increasing concentrations (0.5%, 1.5% and 3.0%) of Intravail[reg], a patented alkylsaccharide transmucosal absorption enhancing agent. The pharmacokinetics of orally delivered (by gavage) octreotide acetate in Intravail[reg] were compared to those of octreotide acetate administered subcutaneously in sodium acetate buffer to male Swiss Webster mice. Oral delivery of octreotide acetate in 0.5% Intravail[reg] significantly enhanced total uptake (1,254.08 ng/ml/min vs. 311.63 ng/ml/min, respectively), serum half-life (52.1 min vs. 3.1 min, respectively), and relative bioavailability (4.0 vs. 1.0, respectively) when compared to delivery by s.c. injection. Higher concentrations of Intravail[reg] did not further enhance uptake, serum half-life, or bioavailability. The results of this study indicate that oral delivery of octreotide acetate in Intravail[reg] is feasible, and is an effective method of administration which significantly improves uptake, bioavailability and half-life when compared to s.c. injection. Thus, oral delivery of octreotide acetate in Intravail[reg] may have significant potential as a novel, non-invasive approach to the treatment of acromegaly and octreotide-mediated symptoms of carcinoid and VIP-secreting tumors in humans.

Sources of Research Support: Grant from the Willard B. Warring Memorial Fund, and by Aegis Therapeutics.

Disclosures: EEM: Chief Scientific Officer, Aegis Therapeutics. Nothing to Disclose: PG

Pub # P1-286

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Evidence That Androgen Synthesis by the Theca-Interstitial Cell Is Regulated by Luteinizing Hormone-Mediated mTORC1 Activation

Author String M Palaniappan, K Menon
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Body Androgens are produced by the theca-interstitial (T-I) cells of the ovary in response to luteinizing hormone (LH) and are transported to the granulosa cells where these androgens are converted to estrogens. Hyperactivity of theca cell function is associated with conditions that lead to anovulatory hyperandrogenism, characteristic of polycystic ovarian syndrome (PCOS). Our previous studies have shown that LH/hCG-activates mammalian target of rapamycin complex 1 (mTORC1) signaling in T-I cells. The present studies were performed to determine whether LH/hCG-mediated mTORC1 signaling plays a direct regulatory role in androgen biosynthesis. T-I cells were isolated from 25 day-old rat ovaries by collagenase digestion and cultured in the presence and absence of mTORC1 inhibitor, rapamycin (20 nM) for 1 h followed by stimulation with hCG (50 ng/ml) or forskolin (10 [μ M]) for additional 24 h. Whole cell lysates were analyzed for androgen biosynthetic enzymes and downstream targets of mTORC1 by Western blot analysis. The results showed that hCG or forskolin treatment resulted in more than two-fold increase in CYP11A1, 3 β -HSD and CYP17A1 as well as S6K1, eIF4E, CREB and SREBP-1a protein expression, whereas rapamycin treatment significantly reduced these responses. HCG or forskolin-induced StAR protein, however, was not affected by rapamycin treatment. Analysis of androstenedione levels in the media revealed that treatment with rapamycin significantly reduced hCG-stimulated androstenedione secretion. To provide further evidence for the role of mTORC1 in androgen biosynthesis, T-I cells were transfected with control siRNA (non-target) or mTORC1 siRNA (target) for 48 h followed by hCG-stimulation for additional 24 h. The effectiveness of siRNA to block mTOR expression was confirmed by Western blot analysis. The results revealed that, as expected, hCG treatment elicited an increase in the expression of steroidogenic enzymes CYP11A1, 3 β -HSD and CYP17A1, whereas siRNA-mediated knockdown of mTORC1 significantly diminished this response to hCG. Furthermore, hCG-induced CREB phosphorylation, a key regulator of steroidogenic enzymes, was abrogated by treatment with rapamycin. Taken together, our results conclusively show that mTORC1 signaling plays a central role in LH/hCG-stimulated CREB activation and androgen biosynthesis in T-I cells.

Sources of Research Support: NIH Grant HD-38424.

Nothing to Disclose: MP, KMJM

Pub # P1-287

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Insufficient Follicular Fluid Vitamin D Levels Are Associated with Anti-Müllerian Hormone Receptor Gene Up-Regulation in Human Luteinized Cumulus Granulosa Cells of Small Follicles

Author String ZO Merhi, A Zapantis, D Berger, S Jindal
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Body **Background:** We have recently shown that serum and follicular fluid (FF) 25 OH-vitamin D levels are highly correlated, and that patients who achieved clinical pregnancies following in vitro fertilization exhibited significantly higher FF 25 OH-vitamin D levels (1). Anti-mullerian hormone (AMH), produced by granulosa cells (GC), is a marker of ovarian reserve. Vitamin D has been shown to upregulate AMH gene expression in human prostate cancer cells (2) but the association of vitamin D with AMH/AMH-receptor (R) expression in GC has not yet been studied. We hypothesize that FF 25 OH-vitamin D levels are associated with AMH/AMH-R gene expression in human luteinized GC.

Materials and Methods: 13 reproductive-aged (age range: 24-45) women who underwent controlled ovarian hyperstimulation followed by oocyte retrieval were enrolled. Mural and cumulus GC from small (SF<14 mm) and large follicles (LF[ge]14 mm) were collected separately. The mRNA was isolated, reverse transcribed and AMH/AMH-R gene expression was quantified using RT-PCR. Relative gene expression was calculated using the $2^{-[\Delta\Delta]CT}$ method with GAPDH as reference gene. 25 OH-vitamin D (n=13) levels were measured from the first large follicle entered during oocyte retrieval. FF 25 OH-vitamin D level >30 ng/mL was defined as [ldquo]replete[rdquo] whereas level <30 ng/mL was defined as vitamin D [ldquo]insufficiency.[rdquo] Mann-Whitney U test and Spearman's rank correlation were used.

Results: Patients with insufficient FF 25 OH-vitamin D levels (n= 7) had significantly higher AMH-R gene expression in the cumulus GC of SF compared to women with replete FF 25 OH-vitamin D levels (n=6) (5.2 ± 0.8 versus 2.8 ± 0.6 respectively; P =0.02). In all participants (with replete and insufficient 25 OH-vitamin D) there was a negative correlation between 25 OH-vitamin D and AMH-R gene expression in cumulus GC of SF (r=-.60, P=0.04) and there was a trend, although did not reach statistical significance, towards a negative correlation between 25 OH-vitamin D and AMH-R gene expression in cumulus GC of LF (r=-.71, P=0.1). There were no significant association between FF 25 OH-vitamin D levels and AMH gene expression.

Conclusion: We report for the first time a possible association between insufficient 25 OH-vitamin D levels within the ovarian follicle and increased cumulus cell gene expression of AMH-R which binds AMH, a clinical marker of ovarian reserve. Further research exploring the association of vitamin D to AMH activity in GC is warranted.

1) Ozkan S, Jindal S, Greenseed K, Shu J, Zeitlian G, Hickmon C et al. Replete vitamin D stores predict reproductive success following in vitro fertilization. *Fertil Steril* 2010;94:1314-9.

2) Malloy PJ, Peng L, Wang J, Feldman D. Interaction of the vitamin D receptor with a vitamin D response element in the Mullerian-inhibiting substance (MIS) promoter: regulation of MIS expression by calcitriol in prostate cancer cells. *Endocrinology* 2009;150:1580-7.

Sources of Research Support: Ferring Pharmaceutical Grant to SJ; Ferring Pharmaceutical Grant to ZM through New England Fertility Society.

Nothing to Disclose: ZOM, AZ, DB, SJ

Pub # P1-288

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title An *In Vitro* Culture System to Retrieve Mature Mouse Oocytes after Activation of Dormant Follicles

Author String J Li, Y Cheng, J Cho, S Takae, Y Sato, A Hsueh
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Body Ovarian tissue preservation and auto-transplantation is an option for women with cancer who want to safeguard their fertility, especially for pre-pubertal girls or women requiring immediate cancer treatment. However, there still exists a risk of metastatic spread to the ovary which may re-introduce aggressive cancer cells to the recent survivor of their diseases. Therefore, it is important to develop in vitro culture methods to generate mature oocytes. Eppig and O'Brien (1)(2) succeeded in setting-up a two-step culture system in which mouse ovarian explants containing primordial follicles were cultured to the secondary stage before subsequent culturing of granulosa-oocyte complexes to generate mature oocytes. Because the efficiency of this approach is low, most other labs continue to use preantral follicle cultures to generate mature oocytes and viable offspring. Our recent data (3) showed that primordial follicles could be activated by a short-term treatment with PTEN inhibitor and phosphatidylinositol-3-kinase (PI3K) activator. Using this approach, we set up a new two-step culture system to generate mature oocytes from mouse primordial follicles. Ovaries from day 3 mice containing mainly primordial follicles were activated with the PTEN inhibitor (bpV(HOpic), 10~30uM) and a PI3K activator (740Y-P, 50~150ng/ml) for 48h. Media were changed and recombinant GDF9 (100ng/ml) was added for subsequent cultures for 11 more days. Analyses of follicle dynamics using serial ovarian sections indicated 2.3-, 1.4-, and 1.8-fold increases in the number of primary, early preantral (90-120 um in diameter), and late preantral (>120um) follicles, respectively. The observed increases of preantral follicles were confirmed by immuno-histochemical staining and immunoblotting using Anti-Mullerian hormone (AMH) antibodies. After 13 days of organ culture, preantral follicles were then dissected out mechanically from activated ovaries and cultured on ovarian stromal cell feeder layer. After 10~12 days of culture on feeder, the diameter of follicles grew from ~110um to ~400um and oocyte diameters increased from ~60um to ~70um. Following in vitro maturation, mature oocytes capable of undergoing germinal vesicle breakdown (GVBD) were found in 70% of cultured intact preantral follicles. The present findings suggest in vitro activation using PTEN inhibitor and PI3K activator is an efficient way to generate mature oocytes after long-term culture of isolated follicles.

- (1) Eppig JJ and O'Brien MJ, Biol Reprod 1996; 54:197
- (2) O'Brien MJ et al., Biol Reprod 2003;68:1682
- (3) Li J et al., Proc Natl Acad Sci U S A 2010;107:10280.

Nothing to Disclose: JL, YC, JC, ST, YS, AH

Pub # P1-289

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Mouse Forkhead L2 (FOXL2) Functions as a Transcriptional Repressor of Key Genes in Granulosa Cell Steroidogenesis and Proliferation

Author String M Pisarska, FT Kuo, I Bentsi-Barnes, G Barlow
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Body The forkhead transcription factor FOXL2 (forkhead box L2) is expressed in granulosa cells of small and medium follicles in the mouse ovary, and human mutations of FOXL2 are associated with premature ovarian failure in Blepharophimosis-Ptoxis-Epicanthus Inversus (BPES) type 1. We have previously shown that human FOXL2 functions as a transcriptional repressor of the Steroidogenic Acute Regulatory (StAR), P450aromatase, P450scc, and cyclin D2 genes, markers of ovarian follicle proliferation and differentiation. In this study, we examined whether mouse FOXL2 also functions as a transcriptional repressor of these genes. We found that mouse FOXL2 represses the activities of the mouse StAR, P450aromatase, P450scc promoters in CHO cells, but does not function as a repressor of the cyclin D2 promoter. Using luciferase-promoter constructs, we identified the minimal mouse StAR, P450aromatase and P450scc promoter regions responsive to regulation by FOXL2. To confirm that mouse FOXL2 controls expression of these genes in granulosa cells we used siRNA to knock down mouse FOXL2 in primary mouse granulosa cell cultures. The mRNA expression levels of mouse StAR, P450aromatase and P450scc, but not cyclin D2, increased significantly following knock down of mouse FOXL2. We then increased the expression levels of FOXL2 by generating a lentiviral construct for mouse FOXL2 and using it to infect primary mouse granulosa cells. Following lentiviral infection, the expression levels of mouse StAR, P450aromatase and P450scc, but not cyclin D2, decreased significantly. These data confirm that, similar to human FOXL2, mouse FOXL2 functions as a transcriptional repressor of key genes in ovarian follicle development.

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Nothing to Disclose: MP, FTK, IB-B, GB

Pub # P1-290

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Germ-Cell-Specific Deletion of Jagged1, a Notch Ligand, Leads to the Formation of Multi-Oocytic Follicles in the Mouse Ovary

Author String DA Vanorny, SM Kilen, KE Mayo
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Body During ovarian development, germ cell syncytia (nests) undergo breakdown as somatic pre-granulosa cells encapsulate individual oocytes to form primordial follicles. In the mouse ovary, this occurs in the early postnatal period. The Notch ligand Jagged1 is expressed within oocytes while the receptor Notch2 is restricted to the granulosa cells of the ovary. This complementary pattern of expression suggests a role for Notch signaling in mediating communication between the germ cell and somatic cells of the forming follicle. To investigate the roles of Notch signaling within the ovary, we used the Cre/loxP system to disrupt the Jagged1 gene specifically within germ cells (floxed mice provided by J. Lewis, London Research Institute, UK; Vasa^{Cre} transgenic mice provided by D. Castrillon, University of Texas Southwestern Medical School). Postnatal day 21 (dpn21) knockout mice (Vasa^{Cre/+}, J1^{fl/-}) had a profound decrease in ovarian Jagged1 mRNA levels, indicating the effectiveness of the disruption. As well, expression of the Notch signaling target Hes-1, which is expressed in granulosa cells, was significantly reduced. Histological examination of the ovaries revealed the presence of numerous multi-oocytic follicles (MOFs), or follicles containing more than one oocyte, within the Vasa^{Cre/+}, J1^{fl/-} ovary. These abnormal follicles were increased 12-fold over the heterozygous control, and some follicles contained as many as 6 oocytes. The persistence of MOFs suggests that nest breakdown is delayed by the disruption of Notch signaling, consistent with earlier studies from our laboratory using pharmacologic inhibition of Notch signaling. The Vasa^{Cre/+}, J1^{fl/-} ovaries also have numerous aberrant follicles with enlarged or degenerating oocytes. The knockout ovaries also exhibit an increase in cell death within the granulosa cells of follicles, as determined using TUNEL staining. Despite these multiple abnormalities, initial studies indicate that the female knockout mice are fertile. Analysis of dpn5, knockout ovaries indicates that MOFs and abnormal follicles are observed at this early time when the initial follicle pool is established. These studies indicate an important role for Jagged1 and Notch signaling in the mouse ovary and in follicle formation and function. Current studies are aimed at better understanding the mechanisms by which loss of Jagged1 impacts granulosa cell function and follicle development.

Sources of Research Support: NIH P01 HD021921 and NIH training grant T32 GM12453.

Nothing to Disclose: DAV, SMK, KEM

Pub # P1-291

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Follicle Fluid of Obese Women Induces Endoplasmic Reticulum Stress in Cumulus-Oocyte Complexes and Blocks Oocyte Maturation

Author String RL Robker, X Yang, LL-Y Wu, M Lane, RJ Norman
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Body The ovarian follicle provides nutrients and hormonal signals critical for normal maturation of the oocyte and its competence to develop into a healthy embryo. Previous studies from our laboratory have shown that the follicular environment of obese women is altered compared to that of moderate weight women; in particular follicle fluid of obese women contains high levels of triglyceride lipid (1). Further, our studies in mice have shown that diet-induced obesity causes the induction of endoplasmic reticulum stress and lipotoxicity pathways in cumulus-oocyte complexes (COCs) (2) and decreased fertilization and oocyte developmental competence (3). The aim of the current study was to determine whether similar events occur in women; namely whether the altered follicular environment of obese women induces endoplasmic reticulum stress in COCs and directly impacts oocyte maturation. Follicle fluid (FF) was obtained during oocyte aspiration from women of known Body Mass Index (BMI). FF with the highest levels of triglyceride and free fatty acids were from obese women (BMI 33 ± 1) while those with the lowest levels were from moderate weight women (BMI 24 ± 0.8). To determine the effect of the lipid-rich obese FF on oocyte maturation, mouse COCs were used as surrogates for human oocytes, and were matured for 16h in media containing FF from either obese women or moderate weight women. Exposure to obese FF caused lipid accumulation in oocytes and increased mRNA expression of the lipid droplet protein perilipin-2. COCs matured in obese FF also had increased expression of 3 distinct markers of endoplasmic reticulum stress (Atf4, Atf6 and Grp78) compared to COCs matured in FF from moderate weight women. Finally, maturation in obese FF profoundly impaired oocyte nuclear maturation: only 26% of oocytes underwent polar body extrusion compared to 87% of oocytes matured in FF from moderate weight women ($P < 0.0001$). FF samples were assayed for adipokines and apolipoproteins to identify factors in obese women that may cause impaired oocyte maturation. Compared to FF from moderate weight women, obese FF contained elevated levels of ApoAI, ApoAII, and leptin, IL-6 and IL-10 cytokines ($P < 0.01$). These results demonstrate that 1) the follicular environment of obese women is markedly different from that of moderate weight women and; 2) that maturation within this environment is detrimental to oocyte; and may contribute to the decreased conception rates observed in obese women.

1. Robker RL, Akison LK, Bennett BD, Thrupp PN, Chura LR, Russell DL, Lane M, Norman RJ. Obese women exhibit differences in ovarian metabolites, hormones, and gene expression compared with moderate-weight women. *J Clin Endocrinol Metab* 2009; 94:1533-1540.
2. Wu LL, Dunning KR, Yang X, Russell DL, Lane M, Norman RJ, Robker RL. High-Fat Diet Causes Lipotoxicity Responses in Cumulus-Oocyte Complexes and Decreased Fertilization Rates. *Endocrinology* 2010.
3. Minge CE, Bennett BD, Norman RJ, Robker RL. Peroxisome proliferator-activated receptor-gamma agonist rosiglitazone reverses the adverse effects of diet-induced obesity on oocyte quality. *Endocrinology* 2008; 149:2646-2656.

Sources of Research Support: National Health & Medical Research Council of Australia grants awarded to RLR, ML, RJN and National Science Foundation of China fellowship awarded to XY.

Nothing to Disclose: RLR, XY, LL-YW, ML, RJN

Pub # P1-292

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title The Regulation of Inhibitory *Smad7* in the Ovary

Author String MB Quezada, J Wang, EA McGee
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Body The TGF β family of growth factors play critical roles in normal ovarian folliculogenesis. Signaling is generally via the Smad family of proteins. Smad7 is an inhibitory Smad that modulates receptor activated Smads and therefore the overall activity of TGF β family signaling. The regulation of Smad7 in the ovary is largely unknown. We have determined that TGF β treatment of granulosa cells isolated from mice results in an average increase of more than 2 fold of Smad7 mRNA ($p < 0.05$). In dose response studies a maximal stimulation is plateaus at 10 ng/mL. The addition of FSH or cAMP activators did not affect the TGF β stimulated increase in Smad7. TGF β treatment also activates a Smad7 promoter/luciferase reporter system transfected into SIGC cells. TGF β treatment (1 ng/ml) results in a 3.8 fold increase in luciferase induction over baseline ($p < 0.05$). Deletion constructs abolish this TGF β generated effect. Further studies are underway to more precisely locate the areas of the promoter responsible for the activation. Granulosa cells that do not express Smad3, maintain the ability to upregulate Smad7 in response to TGF β . Likewise inhibition of Smad3 with a chemical inhibitor does not prevent TGF β stimulation of Smad7 mRNA or promoter activation. However, simultaneous inhibition of Smad2 and Smad3 does reduce TGF β stimulated SMAD7 expression. TGF β is a specific modulator of Smad7 mRNA expression and promoter activity in granulosa cells. TGF β regulation of Smad7 provides an important feedback loop in regulation of Smad signaling in the ovary.

Sources of Research Support: HD034449, HD045700.

Nothing to Disclose: MBQ, JW, EAM

Pub # P1-293

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Regulation of Granulosa Cell Proliferation and Apoptosis by Cyp26b1 and Retinoic Acid in the Mouse Ovary

Author String G Rodriguez, M Demczuk, E Haraelli, K Mayo, J Kipp
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Body Cyp26b1 is a member of the cytochrome P450 family and is an enzyme that degrades the potent morphogen retinoic acid (RA). Cyp26b1 is important for limb, nervous system and bone development. It has also been shown to inhibit germ cell meiosis in the male gonad. We recently demonstrated expression of Cyp26b1 mRNA and protein in granulosa cells of ovarian follicles at all postnatal developmental stages, and discovered that Cyp26b1 gene expression is strongly inhibited by activin. We found that activin, RA and the Cyp26 inhibitor R115866 increased granulosa cell numbers while a pan-retinoic acid receptor (RAR) inhibitor, AGN194310, abolished these stimulatory effects, indicating an involvement of RAR-mediated signaling. To further understand the functions of Cyp26b1 in the mouse ovary, this study was designed to investigate the consequences of Cyp26b1 overexpression or treatment with a Cyp26 inhibitor or RA on granulosa cell proliferation and apoptosis. Granulosa cells were collected from day 21-23 immature mice and cultured for 2 days, and cell proliferation and apoptosis were analyzed using an MTS assay or with Fluorescence Activated Cell Sorting (FACS) after BrdU or Annexin V labeling. Overexpression of Cyp26b1 suppressed granulosa cell proliferation and induced cell apoptosis, while R115866 and RA stimulated cell proliferation and suppressed apoptosis. RA measurements showed that Cyp26b1 overexpression decreased while R115866 increased RA concentrations, suggesting that changes in Cyp26b1 can regulate the RA environment in granulosa cells and have profound biological consequences. RA levels were also increased in granulosa cells treated with activin A and decreased in those treated with the activin antagonist follistatin. These observations are consistent with the suppressive effect of activin on Cyp26b1 expression and the known stimulatory effect of activin on granulosa cell proliferation. Overall, this study provides evidence that Cyp26b1 inhibits while RA promotes granulosa cell growth through regulation of both proliferation and apoptosis, and that the activin and RA pathways may interact to regulate ovarian function.

Sources of Research Support: Grants from DePaul University as well as by NIH Program Project Grant HD21921.

Nothing to Disclose: GR, MD, EH, KM, JK

Pub # P1-294

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Role of microRNA in the Expression of Luteinizing Hormone-Human Chorionic Gonadotropin Receptor mRNA in Rat Ovary

Author String Y Kitahara, K Nakamura, K Kogure, T Minegishi
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Body [Introduction] Recently, it has become increasingly evident that microRNAs (miRNAs), which are made up of approximately 22 nucleotides and noncoding RNAs, are evolutionally well-conserved in diverse organisms and negatively regulate gene expression at the post-transcriptional level. In this study, we investigated whether miRNA is involved in down-regulation of the LH receptor (LHR) in the ovary.
[Methods] For *in vivo* study, PMSG-hCG priming rat ovaries were removed at selected time, and we isolated total RNA from them. A miRNA microarray was carried out to analyze the overall miRNA expression profile while LHR mRNA was down-regulated using these total RNAs. The target miRNAs, which bound to the rat LHR mRNA, were predicted by using the miRBase (<http://www.mirbase.org/>) on the basis of the miRNA microarray results. Based on these results, we examined the time-dependent change of expression of a miRNA by real time RT-PCR. For *in vitro* study, the granulosa cells were obtained from DES priming rats, and we then cultured the granulosa cells. To directly assess whether a miRNA regulates LHR mRNA, we transfected miRNAs or miRNA inhibitor to cultured granulosa cells. We examined time-dependent change of the LHR expression by real time RT-PCR. In addition, the binding site of a miRNA into LHR mRNA 3'-untranslated region (3'-UTR) was identified by the luciferase reporter assay.
[Results] In the miRNA microarray, 23 miRNAs expression has increased after hCG had been stimulated. Combining these results with data from the bioinformative database, the clustering analysis led us to focus on rno-miR-136* for further analysis. So we examined time-dependent change of rno-miR-136* expression in rat ovaries by real time RT-PCR. The miR-136* levels were found to increase 6 hr after hCG administration while LHR mRNA levels were down-regulated. In *in vitro* study, we transfected miR-136* to cultured granulosa cells, demonstrating that LHR mRNA levels were significantly decreased in comparison with those of cells transfected with negative control. In contrast, miR-136* inhibitor antagonized the miR-136* effect, resulted in increasing LHR mRNA levels. Moreover, luciferase assays showed that when rno-miR-136* bound to 2848-2866 in the LHR mRNA 3'-UTR, it inhibited LHR translation.
[Conclusion] From these data, we conclude that miR-136* participates in the mechanism of down-regulation of LHR mRNA, whereby miR-136* forms base pairs with LHR mRNA.

Nothing to Disclose: YK, KN, KK, TM

Pub # P1-295

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Neuromedin U Acts as an Autocrine/Paracrine Regulator That Attenuates Progesterone Production by Luteinizing Theca-Interstitial Cells

Author String T-Y Lin, C-W Luo
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Body Neuromedin U (NMU) has been originally known to control the physiological functions of digestive, nervous and immune systems through its endogenous receptors, NMUR1 and NMUR2. Growing studies have potentially linked the NMU action to development of female reproductive organs. However, no direct evidence has been provided. Using a rat superovulatory model, we found that the NMU transcript level is tightly regulated by gonadotropins. Ovarian microdissection followed by real-time PCR analysis indicated that NMU is expressed moderately in granulosa cells and mainly in the theca-interstitial layer, where it was co-expressed with two NMURs. Primary culture results indicated that the NMU transcript level in granulosa and theca-interstitial cells was increased right after a short-term activation of the cAMP/PKA pathway but decreased dramatically to a basal level in a prolonged activation; such a decrease is partially determined by the uncharacterized nature of the 3' untranslated region of NMU mRNA. Furthermore, NMU treatment attenuated the hCG-induced progesterone production in mature theca-interstitial cells. Such a phenomenon was accompanied by downregulating the levels of LH receptor and hCG-induced steroidogenic genes such as HSD3 β and CYP11a1. Thus, these results suggest that gonadotropin-stimulated NMU is involved in the luteinizing regulation of the ovary.

Sources of Research Support: NCS97-2311-B-010-001-MY3.

Nothing to Disclose: T-YL, C-WL

Pub # P1-296
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)
Title Linkage of Regulators of TGF β Activity in the Human Fetal Ovary to Polycystic Ovary Syndrome
Author String N Hatzirodos, RA Bayne, HF Irving-Rodgers, K Hummitzsch, L Sabatier, S Lee, W Bonner, MA Gibson, WF Rainey, BR Carr, HD Mason, DP Reinhardt, RA Anderson, RJ Rodgers

Body Although not often discussed the ovaries of women with polycystic ovary syndrome (PCOS) show all the hallmarks of increased TGF β activity with increased amounts of the fibrous tissue and collagen in the ovarian capsule or tunica albuginea and ovarian stroma (1). Fetal androgenisation leads to PCOS later in life suggesting that PCOS could have a fetal origin (2). There is evidence of a genetic predisposition to PCOS and genetic studies of PCOS have found linkage with a microsatellite located in intron 55 of the extracellular matrix protein, fibrillin 3 (3). Fibrillins regulate TGF β bioactivity in tissues by binding latent TGF β binding proteins. We therefore examined expression of fibrillins (*FBN*) 1, 2 and 3, latent TGF β binding proteins (*LTBPs*) 1, 2, 3 and 4 and TGF β 1, 2 and 3 in human fetal ovaries in the first and in early and late second trimesters and for comparison in adult stroma/tunica using real time RT-PCR (n = 5-6 per group). *FBN3* was highly expressed in the first trimester and then declined during gestation to reach very low levels in adult ovaries. The expression levels of *FBN3* were as high as *FBN1* and *2* in the first trimester. All four *LTBPs* were expressed during gestation. *LTBP3* and *4* were substantially higher in adult ovaries than fetal ovaries. *TGF β 3* expression levels were relatively uniform in the four groups of ovaries with *TGF β 3* being lower than *TGF β 1* or *2*. We also immunolocalised fibrillin 3 in fetal ovaries at 10, 12, 14, 19 weeks of gestation, and in fetal lung as a positive control. Fibrillin 3 localised as fibres in the stroma between ovigerous cords or nests of oögonia the fetal ovary. Staining was more intense in first trimester ovaries and fainter or not detectable at later stages. The results indicate that TGF β pathways operate during ovarian fetal development, but most importantly we show fibrillin 3 is present in the stromal compartments of fetal ovaries and is highly expressed at a critical stage early in developing human fetal ovaries when stroma is expanding and follicles are forming. These changes in expression of fibrillin 3 in the fetal ovary could lead to a predisposition to develop PCOS in later life.

- (1) Hughesdon PE, *Obstet Gynecol Surv* 1982; 37:59
- (2) Abbott DH et al., *Hum Reprod Update* 2005; 11:357
- (3) Stewart DR et al., *J Clin Endocrinol Metab* 2006; 91:4112

Sources of Research Support: National Health and Medical Research Council of Australia, the University of Adelaide, the Clive and Vera Ramaciotti Foundation, the Wellcome Trust, the National Institute of Health, and the Medical Research Council UK.

Nothing to Disclose: NH, RAB, HFI-R, KH, LS, SL, WB, MAG, WER, BRC, HDM, DPR, RAA, RJR

Pub # P1-297

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Metformin Inhibits Follicle-Stimulating Hormone (FSH) Action in Human Granulosa Cells: Relevance to Polycystic Ovary Syndrome

Author String S Rice, L Pellatt, U Thiruchelvam, Z Jawad, HD Mason
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Body Results of several studies(1)have demonstrated that metformin can induce regular menstrual cycles and increase ovulation in women with polycystic ovary syndrome(PCOS). We have previously shown that part of the efficacy of metformin's actions is due to a direct action on ovarian steroidogenesis, by inhibition of granulosa cell expression and activity of aromatase(2). Metformin inhibited insulin-stimulated aromatase via activation of ERK-1,2 signalling pathway which negatively regulates CYP19 expression. However, the interaction of metformin with other pathways that are involved in aromatase production remain to be elucidated. Chief amongst these is the FSH-stimulated cAMP/PKA pathway. In order to investigate this KGN cells were cultured with 10^{-7} M metformin (16ng/ml), FSH at 5 & 10ng/ml \pm metformin, forskolin (FSK) at 1 & 25[μ]M \pm metformin for 48hrs. Reverse transcribed mRNA was quantified for aromatase and FSHR expression and normalized to L19. To investigate aromatase promoter II (PII) activity, cells were transfected with a PII-specific reporter construct as well as 5ng/[μ] of Renilla expression vector as a transfection control. After serum-starvation, cells were treated as described above except the FSH doses were extended to 1, 2.5 and 20ng/ml. Luciferase reporter assays were carried out using the Dual-Glo Luciferase Assay System. FSH increased aromatase mRNA expression in a dose-dependent manner which was attenuated by the presence of metformin. FSH, especially at 1ng/ml, was able to markedly enhance PII-driven aromatase expression, which was significantly reduced by metformin. However metformin did not decrease FSK-stimulated aromatase levels, indicating that the inhibitory effect of metformin on FSH action maybe upstream of cAMP signalling i.e on the FSH receptor (FSHR). Surprisingly, metformin alone was able to markedly reduce basal FSHR levels. A high FSK dose(25[μ]M)also suppressed FSHR mRNA and interestingly the addition of metformin reduced FSHR mRNA levels even further. In the liver, metformin has been shown to act at a cellular level to phosphorylate CBP (CREB-binding protein) and bring about the disassembly of the CREB transcriptional complex(3), and it remains to be determined if a similar mechanism exists in granulosa cells. To conclude, metformin reduced FSH-stimulated aromatase expression and FSHR levels. This is of concern given its widespread use in anovulatory PCOS.

(1) Palomba S et al., Endocrine Rev 2009; 30:1

(2)Rice S et al., Endocrinology 2009;150:4794

(3)He L et al.,Cell 2009; 137:635

Sources of Research Support: Wellcome Trust (WT073572MA, 081420/Z/06/Z)to SR,HDM.

Nothing to Disclose: SR, LP, UT, ZJ, HDM

Pub # P1-298

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Expression of miRNAs in the Adipose Tissue of Lean Patients with PCOS

Author String Y-H Chen, S Heneidi, R Azziz
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Body **BACKGROUND:** Polycystic ovary syndrome (PCOS) is characterized by a complex set of symptoms, with numerous genes contributing to its development. MicroRNAs (miRNAs) regulate numerous biological mechanisms, but no studies to date have investigated miRNA regulation in PCOS. Examining adipose tissue from PCOS patients with a lean phenotype allows for insulin resistant pathways to be studied without being masked by normal insulin resistance associated with high BMIs.
AIM: To investigate the relationship between miRNA expression and PCOS, we compared miRNA expression in adipose tissue from lean women with and without PCOS.
METHODS: Subcutaneous adipose tissue was collected from patients with BMIs less than 25 and matched controls (n = 3 for each group). Using the affymetrix GeneChip miRNA Array, we measured the expression of over 4000 microRNAs in adipose from lean PCOS. Analysis was performed with Ingenuity Pathway Analysis (IPA) software.
RESULTS: We found 120 significantly differentiated microRNAs (p value < 0.05) with changes of at least 2 fold. Of these, miRNAs associated with reproductive system dysfunction (n=11) and genetic disorders (n=14) were significantly regulated. Predicted target genes for these miRNAs include insulin signaling pathway molecules FOS, p38 MAPK, 17 beta-estradiol, PI3K, DLC1 and TNF-alpha. Most significantly regulated miRNAs include miR-141, which targets DLC1, and miR-183, which is insulin responsive and has been linked with ovarian cancer. Other significantly regulated miRNAs such as miR-335, miR-338, and miR-27a (PCOS vs control fold change of -8.6, 2.0, and 2.8, respectively) have been implicated in lipogenesis, lipid metabolism, cholesterol homeostasis, and fatty acid metabolism.
CONCLUSION: The expression patterns of numerous miRNAs are differentially expressed in adipose tissue of lean patients with PCOS. These miRNAs are associated with pathways involving reproductive system dysfunction, insulin signaling, and lipid metabolism. In lean PCOS, miR-141 and miR-183 were the most significantly regulated, suggesting a role for these miRNAs in the pathophysiology of PCOS. Examining specific gene targets may lead to PCOS specific biomarkers and greater understanding of adipose tissue metabolism and regulation in PCOS.

Nothing to Disclose: Y-HC, SH, RA

Pub # P1-299

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Glucocorticoid Receptor Polymorphisms in Polycystic Ovary Syndrome and HAIR-AN Syndrome: Clues for Clinical Management

Author String GA Maciel, RP Moreira, DD Bugano, SA Hayashida, JA Marcondes, LG Gomes, TS Bachegea, BB Mendonca, EC Baracat
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Body
Introduction: Polymorphisms of the glucocorticoid receptor (GR) have been implied in cortisol sensitivity. Previous studies have suggested that cortisol function is dysregulated in PCOS. HAIR-AN syndrome share a series of features with PCOS and might serve as model of metabolic influence of GR polymorphism in PCOS
Objective: To determine the prevalence of three single-nucleotide polymorphisms (SNPs) of GR gene, A3669G, BCL I, and N363S, and among women with PCOS and HAIR-AN syndrome and to study the association between those polymorphisms and clinical and metabolic features, namely menstrual cycles pattern, hirsutism score, androgen levels, body mass index (BMI), insulin sensitivity, and prevalence of metabolic syndrome.
Methods: One hundred eleven (111) women aged 15-39 years (mean 24.9±5.1) were studied. Ninety seven (97) presented PCOS and fourteen (14) had HAIR-AN syndrome, both diagnosed according to AES-PCOS guidelines. The A3669G, ER22/23EK, BCLI, and N363S GR polymorphisms were screened by direct sequencing in DNA samples extracted from leukocytes, using standard methods.
Results: The A3669G, BCLI, and N363S variants were found in 12.1%, 23.8%, and 0.5% of the 222 alleles, respectively. All of them presented Hardy Weinberg equilibrium. A mutated genotype of A3669G in PCOS subjects (less severe cases) reached almost 25% in comparison to 7% in HAIR-AN patients (more severe cases). Menstrual irregularities were present in 93.75% of PCOS and 100% HAIR-AN subjects (p=0.06), BM were also not different among groups (p=0.26). Basal levels of insulin, $HOMA \geq 2.7$, and abnormalities in OGTT were more evident in HAIR-AN syndrome patients (p=0.002, 0.001 and 0.001, respectively). The presence of metabolic syndrome was higher in HAIR-AN group (57.14 vs 26.8% p=0.02) as well as median testosterone levels (p=0.04). Hirsutism score did not differ between groups (p=0.12).
Conclusion: GR polymorphisms A3669G, BCLI are fairly prevalent in Brazilian hyperandrogenic women and A3669G mutated genotype was associated with less severe metabolic profile, and might be taken in account in the future, regarding health care in PCOS subjects.

Nothing to Disclose: GAM, RPM, DDB, SAH, JAM, LGG, TSB, BBM, ECB

Pub # P1-300

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Dietary Vitamin D₃ Deficiency Creates a PCOS-Like Reproductive Phenotype in *Cyp27b1* Null Female Mice

Author String CL Dicken, J Shu, D Israel, G Neal-Perry
Albert Einstein College of Medicine, Bronx, NY; Albert Einstein College of Medicine, Bronx, NY; Albert Einstein College of Medicine, Bronx, NY

Body Polycystic ovary syndrome (PCOS) is a common endocrine disorder among reproductive aged women characterized by oligo-ovulation and menstrual irregularities. There is accumulating evidence that vitamin D₃ (VitD₃) deficiency is highly prevalent in women with PCOS. To determine if prepubertal VitD₃ deficiency results in a PCOS-like reproductive phenotype, we evaluated reproductive cycles of VitD₃ deficient female transgenic *Cyp27b1* null mice (*Cyp27b1* encodes for 1 α -hydroxylase, the enzyme required to convert 25(OH) VitD₃ to the active 1,25(OH)₂ VitD₃ form). Pups from heterozygous matings were weaned on postnatal day 21 and randomized to either a VitD₃ deficient or sufficient diet. *Cyp27b1* null and WT siblings fed a (D+) diet served as genotype and diet controls, respectively. VitD₃ deficient *Cyp27b1* null mice exhibited significantly prolonged estrous cycles characterized by extended periods of diestrus 1 (P<0.05). VitD₃ deficient *Cyp27b1* null mice also spent significantly less time in proestrus and estrus (P<0.05). The ovaries collected during diestrus from WT mice and *Cyp27b1* null mice wean onto a VitD₃ sufficient diet exhibited corpora lutei and folliculogenesis at various stages of development. In contrast, the ovaries of VitD₃ deficient *Cyp27b1* null mice exhibited arrested folliculogenesis and no corpora lutei. When treated with exogenous gonadotropins and human chorionic gonadotropin, VitD₃ deficient *Cyp27b1* null mice released more oocytes into the oviduct than VitD₃ sufficient WT and *Cyp27b1* (P<0.05). The reproductive phenotype of VitD₃ deficient *Cyp27b1* null mice resembles that of women with PCOS; VitD₃ deficient *Cyp27b1* null mice exhibit irregular estrous cycles with infrequent ovulation characterized by arrested folliculogenesis. In addition, VitD₃ deficient *Cyp27b1* null mice exhibit a much more robust response to exogenous gonadotropins than control mice, a clinical outcome frequently seen in patients with PCOS undergoing controlled ovarian hyperstimulation. These findings suggest that prepubertal VitD₃ deficiency may contribute to the development of PCOS and abnormal hypothalamic-pituitary-ovarian physiology.

Nothing to Disclose: CLD, JS, DI, GN-P

Pub # P1-301

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Evidence for Association of Chromosome 2p16.3 Polycystic Ovary Syndrome (PCOS) Susceptibility Locus in Affected Women of European Ancestry

Author String P Mutharasan, E Galdones, B Penalver Bernabe, OA Garcia, LD Shea, TK Woodruff, RS Legro, A Dunaif, M Urbanek
Northwestern University Feinberg School of Medicine, Chicago, IL; Northwestern University Feinberg School of Medicine, Chicago, IL; Northwestern University, Evanston, IL; MS Hershey Medical Center, Hershey, PA

Body
Background: PCOS is a complex disease. Previous genetic analyses have been based on candidate gene screens. A genome-wide association study, which is an unbiased interrogation of the entire genome, in Chinese women with PCOS identified the genomic region on 2p16.3 containing the genes for luteinizing hormone/choriogonadotropin receptor (LHCGR) and follicle stimulating hormone receptor (FSHR) as a potential susceptibility locus for PCOS (1). However, ethnic/racial differences in susceptibility variants associated with complex genetic diseases occur. To determine whether this region was also associated with PCOS populations of European ancestry, we tested for association between PCOS and variants in LHCGR and FSHR in a US cohort of European ancestry.
Methods: A case-control candidate gene analysis was performed in 976 Caucasian cases diagnosed by NIH criteria and 977 Caucasian control women. As part of a larger analysis of genes differentially expressed in cultured mouse follicles within permissive (soft) and non-permissive (rigid) three dimensional hydrogel environment (2), we tagged the entire coding region of LHCGR and FSHR genes plus 20 kb upstream and downstream of each gene to be informative for minor allele frequency > 0.05 and $r^2 > 0.8$ with 94 haplotype-tagging SNPs and 2 coding SNPs.
Results: We found strong evidence for association with rs10495960 ($p = 0.0008$), which maps within the first intron of LHCGR and the last exon of GTF2A1L, where it encodes an alanine to threonine missense variation. GTF2A1L is expressed in the testis and encodes an uncharacterized protein. Although we did not genotype rs13405728, the marker with the strongest evidence for association in the PCOS GWAS (1), we genotyped three markers (rs35960650, rs2956355, and rs7562879) within 5kb of rs13405728. None of these markers were associated with PCOS in our study. However, since rs10495960 and rs13405728 map to a region with extensive linkage disequilibrium, it is likely that the two SNPs are tagging the same causal variant. We did not detect any evidence for association within FSHR.
Conclusions: We replicated the PCOS susceptibility locus mapping to chr. 2p16.3 identified by Chen et al in a Chinese PCOS cohort. These findings suggest that this region contains a general PCOS susceptibility locus that is important to the etiology of PCOS in women of both Asian and European ancestry. Further studies are needed to determine whether LHCGR or GTF2A1L is the PCOS susceptibility gene.

(1) Chen ZJ et al., Nat Genet 2010; 43:55-59
(2) Woodruff TK and Shea LD, J Assist Reprod Genet 2010; in press

Sources of Research Support: NIH grant 54HD041857 from the National Institute Of Child Health And Human Development awarded to EG, BPB, LDS, TKW, AD and MU; NIH grant R01HD057450 awarded to OAG and MU; NIH grant P50HD044405 from the National Institute Of Child Health And Human Development awarded to AD and MU; American Diabetes Association Career Development Award 7-09-CD-13 awarded to MU.

Nothing to Disclose: PM, EG, BPB, OAG, LDS, TKW, RSL, AD, MU

Pub # P1-302

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Strong and Positive Association of Endothelin-1 with Advanced Glycated End Products in PCOS

Author String S Livadas, F Economou, H Kandaraki, C Christakou, S Palimeri, H Palioura, C Piperi, A Papavasileiou, D Panidis, E Diamanti-Kandarakis
University of Athens, Medical School, Athens, Greece; University of Athens, Medical School, Athens, Greece; Second Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Thessaloniki Greece

Body Introduction: Advanced Glycated End products (AGE's) seem to play a major role in the pathophysiology of several diseases and tissue degradation. Their mechanism of action on endothelial cells has not been fully elucidated yet, but oxidative stress is a key-player in this process. AGE's binding to their receptor (RAGE) leads to oxidative stress increment, whereas AGE's production is induced from oxidative stress. Increased levels of oxidative stress activate the intracellular NF-kB pathway in endothelial cells, leading to Endotelin-1 (ET-1) production. It has been shown that in diabetic endothelial cells in vitro, AGE -RAGE interaction results in NF-kB-dependent ET-1 induction.
This relationship has not been studied in normoglycemic women with PCOS.
Aim of the study: To assess ET-1 and AGE's values in women with Polycystic Ovary Syndrome (PCOS) and control women and to investigate any potential relationship among them. PCOS serve as an ideal group for this study since both AGEs and ET-1 have been reported to be elevated in this syndrome.
Results: Data from 75 lean women with PCOS and 25 controls, matched for age (25,05±4,90 vs. 27,02 ±4,99yrs, p>0.05), BMI (22,15±1,78 vs. 22,35±1,50Kg/m², p>0.05) and WHR (0,74±0,05 vs. 0,71±0,03, p>0.05) were analyzed. ET-1 (1,55±0,13 vs. 0,37±0,10fmol/l, p<0.001) and AGEs (8,34±1,81 vs. 5,77±0,78U/ml, p<0.001) levels were significantly higher in the PCOS group, respectively. Additionally, FG-score, androgens and follicle count were significantly higher in PCOS group, while glucose, insulin levels and HOMA-IR were comparable among groups. ET-1 was highly correlated with AGES (r:0,54, p<0.001), PCOS existence (r:-0,48, p<0.001), Testosterone (r: 0,38, p<0.001), [Delta]4 (r: 0,41, p<0.001) and FAI (r: 0,21, p<0.05). Linear regression analysis showed that ET-1 is highly associated positively only with AGES (β: 0,22 p<0.001).
Conclusions: These data suggest for the first time that ET-1 levels are positively and strongly associated with AGE's, in women with PCOS, suggesting that elevated serum AGE's may be linked with ET-1 production and that both may interact in the cardiovascular abnormalities observed in these women. However, more studies have to be carried out to further investigate the potential pathophysiological mechanism and the potential clinical implications.

Nothing to Disclose: SL, FE, HK, CC, SP, HP, CP, AP, DP, ED-K

Pub # P1-303

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Metformin Inhibits Human Granulosa Anti-Müllerian Hormone Production: An Additional Benefit in Treatment of Women with Polycystic Syndrome?

Author String LJ Pellatt, M Garcia De La Huerta, S Rice, HD Mason
St George's, University of London, London, UK; St George's, University of London, London, UK

Body Metformin is commonly used to treat women with polycystic ovary syndrome (PCOS) and in many cases this is irrespective of the presence of insulin resistance. Metformin has been shown to improve menstrual frequency, insulin sensitivity and androgen levels and we have shown direct inhibition of aromatase mRNA expression and activity. Within the ovary the 'ovarian reserve' factor anti-Müllerian hormone (AMH) is produced solely by the granulosa cells and is overproduced by these cells in the polycystic ovary (PCO). In a number of studies, metformin was also shown to reduce circulating levels of AMH. This was attributed to a new cohort of follicles growing within a normalised androgen and insulin environment. Our aim was to examine a possible further action of metformin on ovarian function by determining whether metformin directly affects AMH expression and protein production in human granulosa cells.

KGN cells (a granulosa tumour cell line), were treated with metformin (10-7M and 10-4M) alone and in the presence of insulin for 24 and 48hrs. AMH expression was determined by real time quantitative PCR (qPCR). Levels of AMH protein produced by granulosa luteal cells treated with metformin for 48hrs were measured by ELISA (DSLabs). AMH mRNA expression after 48hrs was significantly reduced by metformin treatment alone and in the presence of insulin at both doses, (ANOVA p=0.0005). Insulin alone had no effect on AMH expression. After 48hrs 10-7M metformin reduced AMH protein by 65%. The reduction in AMH mRNA expression by metformin at both 10-7M and 10-4M was in the presence of low concentrations of insulin (0.1-1ng/ml), than with higher doses (10-100ng/ml).

In summary, metformin treatment inhibited AMH mRNA expression and protein production in granulosa cell: both alone and in the presence of insulin. During folliculogenesis AMH production is highest in small antral follicles and reduces as the follicle size increases. At the size at which a follicle becomes selected, AMH levels are very low or undetectable, however in women with anovulatory PCOS, the granulosa cells produce 75x more AMH which may contribute to abnormal ovarian function. Treating a subset of women with PCOS high AMH production with metformin may improve their ovulatory status.

Sources of Research Support: MRC project grant to HDM.

Nothing to Disclose: LJP, MGDH, SR, HDM

Pub # P1-304

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Sex Steroid Concentrations Influence the Differential Abundance of Proteins in Omental and Subcutaneous Adipose Tissue

Author String M Insenser, R Montes, JL San-Millan, HF Escobar-Morreale
Hospital U Ramón y Cajal, Universidad de Alcalá, IRYCIS, CIBERDEM, Madrid, Spain; Hospital U Ramón y Cajal, Universidad de Alcalá, IRYCIS, CIBERDEM, Madrid, Spain

Body Background: Polycystic Ovary Syndrome (PCOS) is frequently associated with insulin resistance and obesity, specifically with a predominantly abdominal distribution of body fat. Mounting evidence indicates that androgen excess is a major contributor to the predominantly visceral disposition of body fat in these women. The identification at the tissue level of new proteins involved in the pathogenesis of PCOS is of great interest for the development of more precise diagnostic techniques and the identification of new therapeutic targets. Objective: The aim of the present study is to identify proteins differentially expressed in adipose tissue related to sexual steroid concentrations, by applying two-dimensional differential in gel electrophoresis (2D-DIGE). Methods: Subcutaneous and omental adipose tissue was obtained from 21 morbidly obese patients, including 7 non-hyperandrogenic premenopausal women (age 35.5 ± 6.2 yr; BMI 55.7 ± 2.5 kg/m²), 7 women with PCOS (age 30.4 ± 5.6 yr; BMI 50.8 ± 6.6 kg/m²) and 7 men (age 33.6 ± 6.5 yr; BMI 49.7 ± 6.2 kg/m²). Proteins from adipose tissue were extracted by using a homogenizer. 2D Clean Up Kit was used to remove interfering components. We separated the samples by two-dimensional electrophoresis using immobilized pH gradient (IPG) strips (24 cm, pH 4-7). Results: The analysis by 2D-DIGE of the protein extracts (n=42) is ongoing. Preliminary results to optimize 2-DE experimental conditions show an increase of number of proteins and focusing capacity using the 4-7 pH range versus 3-11 pH range. Previous proteomic studies revealed posttranslational modifications in 15 proteins in omental adipose tissue of PCOS patients and four proteins in depleted plasma proteome of these patients. With this study we expect confirm these earlier candidates and identify more differences between both tissues in non-hyperandrogenic and PCOS women and men. Protein spots showing significant differences between patients and controls will be identified, excised, trypsin-digested and analyzed by matrix-assisted laser-desorption ionization time-of-flight time-of-flight. Conclusion: The precise identification of proteins with differential abundance will provide some insight into the molecular events in visceral and omental adipose tissue associated with sexual steroid concentrations. A further characterization of these differences is necessary to evaluate the possible involvement of subcutaneous and omental adiposity in the development of PCOS.

Sources of Research Support: Grants FIS PI 080944, and CIBER Diabetes y Enfermedades Metabólicas (CIBERDEM) CB07/08/0005 from the Spanish Ministry of Science and Innovation, Instituto de Salud Carlos III, MCINN.

Nothing to Disclose: MI, RM, JLS-M, HFE-M

Pub # P1-305

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Infertile DIO Female Mice Exhibit Tissue-Specific Differences in Insulin-Activated IRS Signaling

Author String S Wu, K-S Shim, K Brothers, S Divall, F Wondisford, A Wolfe
Johns Hopkins University School of Medicine, Baltimore, MD

Body The prevalence of reproductive dysfunction has increased in recent years due to the increasing rate of obesity related diseases such as type 2 diabetes and polycystic ovary syndrome (PCOS). 10% of women exhibit infertility and PCOS accounts for 75% of infertility in reproductive age women. We developed a novel mouse model of diet induced obesity (DIO) that results in infertility and serves as a mammalian model of human PCOS. In this model, cells of the reproductive axis, located in the pituitary and the ovary, are sensitive to the effects of the elevated insulin levels. Basal levels of pAKT in the pituitary and ovary were significantly elevated in DIO mice compared to age matched lean mice. While injection with 0.5, 1 and 1.5 U/kg BW of insulin produced elevated pAKT in energy storage tissues such as liver, muscle of lean mice, only the 1.5 U/kg BW insulin dose produced elevated pAKT in the pituitary and ovary when compared to PBS injected lean wild type mice. In DIO mice, the pituitary and ovary showed elevated pAKT levels in response to insulin while the energy storage tissues exhibited resistance. To explore further the reasons why the pituitary and the ovary maintained insulin sensitivity in the obese state, we measured activation of the insulin receptor substrate proteins (IRS1 and IRS2). Our data suggest that IRS2 mediates the effects of insulin in the pituitary since insulin treatment resulted in elevated pTyr-IRS2 levels in both lean and DIO mice, while pTyr-IRS1 levels are not appreciably changed by insulin treatment in lean or DIO mice. Basal level of pTyr-IRS2 in the pituitary was significantly elevated in DIO mice (1.4±0.1 fold relative to lean mice). In contrast, both IRS1 and 2 may mediate insulin signaling in the ovary since basal levels of both pTyr-IRS1 and pTyr-IRS2 in the ovary were significantly elevated in DIO mice, 1.5±0.15 fold and 1.6±0.19 fold respectively, compared to age matched basal lean mice, and 1.5 U/kg BW insulin stimulated a significant increase in ovary pTyr-IRS1 and pTyr-IRS2 in DIO mice. The liver and muscle of DIO mice exhibit impaired insulin response of both IRS1 and IRS2. These preliminary data suggest that there are differences at this proximal level of the insulin signaling pathway between the ovary and pituitary and the energy storage tissues that become insulin resistant in obesity. Retained sensitivity of the pituitary and ovary to the effects of insulin may contribute to the pathophysiology of PCOS in humans.

Nothing to Disclose: SW, K-SS, KB, SD, FW, AW

Pub # P1-306

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Development of a Mouse Model with Polycystic Ovary Syndrome

Author String L van Houten, A Mcluskey, P Kramer, B Karels, APN Themmen, JA Visser
Erasmus MC, Rotterdam, Netherlands

Body Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women in their reproductive age. Based on the Rotterdam criteria PCOS is defined by two out of the following three criteria: hyperandrogenism, oligo/anovulation, and polycystic ovaries. The etiology of PCOS is unknown, although the failure in dominant follicle selection leading to an accumulation of small antral follicles suggests aberrant FSH sensitivity. In addition, PCOS patients are often abdominal obese, which can lead to insulin resistance. The subsequent increased insulin levels stimulate the ovary to further increased androgen production. Thus, the elevated androgen and insulin levels may result in a detrimental vicious circle between ovary and adipose tissue. Our aim was to develop a mouse PCOS-like model, allowing the future use of transgenic mouse models to study the interaction between ovarian and adipose function in PCOS.

Prepubertal female mice received a 60 days or 90 days continuous release pellet containing the non-aromatizable androgen dihydrotestosterone (DHT) or vehicle. At the end of the treatment period vaginal smears were taken to detect cycle abnormalities. Ovaries were collected to determine the presence of cystic follicles. To determine an effect on metabolism, body weight was measured and fat depots were morphologically analyzed. An Intraperitoneal Glucose Tolerance Test (IPGTT) was performed as a measure for insulin resistance.

DHT treatment for 60 days did not result in differences in reproductive and metabolic characteristics compared to vehicle-treated mice. In contrast, 90 days DHT-treated mice were in continuous an-estrous, suggesting a lack in ovulations, which was confirmed by the absence of corpora lutea in their ovaries. Antral follicles of 90 days DHT-treated mice had a cyst-like structure and there was an increase in the number of atretic follicles, similar to women with PCOS. Also metabolic abnormalities were present. The 90-days DHT-treated mice had significantly higher body weights than vehicle treated mice. In addition, fat depots of DHT-treated mice displayed an increased number of adipocytes of increased size. Furthermore, blood glucose level: during IPGTT were higher, suggesting that these mice are glucose intolerant. We conclude that DHT treatment for 90 days in mice results in a metabolic and reproductive phenotype resembling the phenotype found in PCOS women.

Nothing to Disclose: LvH, AM, PK, BK, APNT, JAV

Pub # P1-307

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title Apolipoprotein A-1 Mimetic Treatment of Ovarian Dysfunction in Two Mouse Models of Polycystic Ovary Syndrome

Author String LB Nedorezov, AR Dowling, JS Marino, X Qiu, JW Hill
University of Toledo, Toledo, OH

Body Polycystic ovarian syndrome (PCOS) is a leading cause of female infertility. It has recently been demonstrated that apolipoprotein A-1 (ApoA-1), the main structural protein in high-density lipoprotein particles, is downregulated in PCOS patients. Cholesterol acts as the initial substrate for the biosynthesis of steroid hormones, and ApoA-1 is required for its transport into the ovary. Hence, reduced levels of ApoA-1 in these cells may disturb normal steroidogenic pathways. We hypothesize that treatment with an ApoA-1 mimetic can ameliorate ovarian dysfunction by directly improving steroidogenesis. Given that excess body weight contributes to the infertility of many PCOS patients, we used the obese (*ob*) mouse as a model in our initial studies. ApoA-1 mimetic 4F or vehicle was administered to mice intraperitoneally for two weeks in a cross-over design. NMR, fasting glucose readings and blood samples were collected at the end of each two-week treatment period. Vaginal cytology of the mice was monitored daily, and body weight and food consumption were measured weekly. Tissues were collected from all euthanized mice. The results show that 4F altered fat deposition, as well as glucose and cholesterol levels in treated mice. However, 4F failed to restore estradiol levels or improve estrous cycles in these mice. Given that leptin deficiency in the *ob* mice complicates interpretation of these results, we have followed up on these studies by examining the effects of 4F treatment in a DHEA-treated mouse model of PCOS.

Sources of Research Support: R00HD056491.

Nothing to Disclose: LBN, ARD, JSM, XQ, JWH

Pub # P1-308
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)
Title Insight into Fetal Androgen Concentrations from Newborn Scalp Hair in Male and Female Rhesus Monkeys
Author String DH Abbott, AK Edwards, AT Beine, DA Dumesic, S Jacoris, AJ Conley
University of Wisconsin, Madison, WI; University of Wisconsin, Madison, WI; University of California, Los Angeles, CA; University of California, Davis, CA

Body Fetal androgen excess in animal models reliably induces polycystic ovary syndrome (PCOS)-like traits in exposed females (1). Confirmation of fetal androgen excess in girls born to women with PCOS, however, has not been forthcoming because of the inherent risks of obtaining blood samples during fetal development. In this regard, scalp hair hormone content may provide a non-invasive method for determining fetal androgen exposure, as steroid hormones remain within growing hair for 3-6 months (2). Since fetal scalp hair develops at mid-gestation, scalp hair at birth has the potential to provide an estimate of fetal androgen exposure. In an initial study to determine whether the androgen content of scalp hair in newborns can differentiate gestational androgen exposure of male from female fetal rhesus monkeys, hair was shaved from approximately 3cm² of scalp in 6 male and 6 female neonates on postnatal day 1 following vaginal delivery. A contemporary blood sample was taken without anesthesia from the neonate's femoral vein. Hair samples were washed before grinding (3) and androgen determinations were made from hair and serum samples by LCMS (4). While hair androstenedione (A) concentrations were greater (p<0.05) in male (32 [22, 57] pg/mg hair; median[25, 75% ile]) compared to female (13 [12, 24] pg/mg) neonates, and the A/DHEA ratio was increased (p<0.05) in males, neonatal hair concentrations for testosterone (T) and DHEA, and the T/A ratio, were comparable (p<0.4) between the sexes (T: male 3 [3, 11] vs. female 2 [1, 5]; DHEA: male 432 [350, 942] vs. female 372 [203, 617] pg/mg). In contrast, postnatal day 1 serum A levels were greater (p<0.04) in female (1.5 [0.9, 1.7] ng/ml) than male (0.7 [0.5, 0.8] ng/ml) neonates, as were (p<0.04) serum levels of DHEA (female: 6.1 [5.6, 7.8]; male: 4.9 [4.0, 5.7] ng/ml). Neonatal serum T levels were comparable (p<0.5) between the sexes (female 0.4 [0.3, 0.6]; male: 0.9 [0.1, 2.7] ng/ml), as were the T/A and A/DHEA ratios (p<0.4). In newborns, androgen determinations of scalp hair may be better than those of blood in detecting greater fetal androgen exposure in male than female rhesus monkeys. Therefore, androgen determination of newborn scalp hair holds promise for identifying infants previously exposed to fetal androgen excess.

- (1) Abbott DH et al., *Am J Primatol.* 2009; 71:776
- (2) Kirschbaum C et al., *Psychoneuroendocrinology.* 2009; 34:32
- (3) Davenport MD et al., *Gen Comp Endocrinol.* 2006; 147:255
- (4) Abbott DH et al., *Biol Reprod.* 2008; 79:154

Nothing to Disclose: DHA, AKE, ATB, DAD, SJ, AJC

Pub # P1-309

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Ovaries & Polycystic Ovary Syndrome (1:30 PM-3:30 PM)

Title mRNA Expression of AMH, FSHR and NF[κ]B in Growing Follicles of Female Lambs Prenatally Exposed to Testosterone

Author String PP Rojas-Garcia, MP Recabarren, S Palma, A Carrasco, M Maliqueo, T Sir-Petermann, SE Recabarren
Universidad de Concepcion, Chillan, Chile; Universidad de Chile, Santiago, Chile

Body Prenatal exposure to excess androgens results in fetal programming in different organs, such as the ovaries. In these, a defect in the mechanism of follicle selection generates an increased number of growing follicles, as in the PCO syndrome. Changes in the expression of ovarian anti-müllerian hormone (AMH) and FSH receptor (FSHR) may be underlying this abnormal follicular recruitment. Therefore, we studied the gene expression of these factors by realtime PCR in preantral follicles of 4-weeks-old female lambs prenatally exposed to excess testosterone (EPT group) and control lambs. Twice a week intramuscular injections of 30 mg testosterone propionate from day 30 to 90 and 40 mg from day 90 to 120 of sheep pregnancy was the prenatal androgen treatment. Control pregnant sheep received only the vehicle. One ovary was left for histologic analysis and the other was left for molecular biology analysis. Ovaries were weighted before follicle separation. Follicles were separated according to the following sizes: less than 0.5 mm and from 0.5 to 1.0 mm (preantral follicles). In order to obtain a proper quantity of RNA for later extraction, follicles of lambs from each group were pooled. Pool of follicles < 0.5 mm contained 63 follicles in the EPT group and 28 follicles in the control group. Pool of follicles 0.5 to 1.0 mm contained 41 follicles in EPT group and 39 follicles in the control group. Ovaries weight was similar at the time of surgery; 318 ± 0.07 mg in EPT group and 264 ± 0.12 mg in control group. However, a difference in the expression of the factors studied was revealed. In the pool of follicles < 0.5 mm, the expression of the FSHR was slightly higher in the EPT group (7.7% above control), while AMH and NFKappaB expression (a transcription factor for AMH) were 50% and 73% lower than control, respectively. The pool of follicles 0.5 to 1.0 mm instead showed a 73% more expression of the FSHR, however AMH turned to be slightly higher than control (2% above), while NFKappaB remained lower (67% of the control expression). These results are in agree with others showing a dysregulation of the paracrine environment governing folliculogenesis in ovaries of females prenatally exposed to androgens as in the PCO syndrome. The lower expression of AMH may result in a increased number of preantral follicles in this model. The role of FSH levels and other transcription factors such as AP2 as well as histologic features in this animal model are still under study.

Sources of Research Support: Fondecyt Grant 1090031.

Nothing to Disclose: PPR-G, MPR, SP, AC, MM, TS-P, SER

Pub # P1-310
Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)
Title Rapid Onset of Efficacy of Desvenlafaxine for the Treatment of Moderate to Severe Vasomotor Symptoms in a Pooled Analysis of 4 Studies in Postmenopausal Women

Author String CJ Guico-Pabia, R-F Cheng, W Bao
Pfizer Inc, Collegeville, PA

Body **Background/Objective:** Amelioration of vasomotor symptoms (VMS) by hormonal therapy in postmenopausal women is pronounced but with a delayed onset of 4 weeks or longer.¹ Hormone-free therapy with desvenlafaxine (administered as desvenlafaxine succinate) has previously been demonstrated to be effective in treating VMS.²⁻⁴ Here we describe the onset of efficacy of desvenlafaxine versus placebo for treatment of moderate to severe VMS in postmenopausal women.

Methods: Data were pooled from 4 double-blind, placebo-controlled trials of postmenopausal women with [ge]50 moderate-to-severe hot flushes (HFs) per week. Women were randomized to receive (1) placebo or desvenlafaxine 50, 100, 150, or 200 mg/d in a 52-week trial (N=707), (2) placebo or desvenlafaxine 100 or 150 mg/d in a 26-week trial (N=567), (3) placebo or desvenlafaxine 100 or 150 mg/d in a 12-week trial (N=458), and (4) placebo or desvenlafaxine 100 mg in a 52-week trial (N=396 [efficacy substudy population]). Onset of efficacy was defined as the first day of 3 consecutive days of [ge]50% reduction in number of daily moderate and severe HFs, and was evaluated from either baseline or from the end of titration to the desvenlafaxine 100 mg dose (ie, point at which patients received the recommended therapeutic dose of 100 mg). Time to onset of efficacy was examined using the Cox Proportional hazard model, with treatment, baseline value, and study in the model.

Results: The analysis included 2,009 randomized patients who took at least 1 dose of study medication and had HF data (primary efficacy variable) at both baseline and on-therapy periods. The population was 86% white with mean \pm SD age 53.6 \pm 4.9 y; BMI 27.2 \pm 4.6 kg/m²; time since natural menopause (with uterus) 5.8 \pm 6.0 y; time since surgical menopause 10.7 \pm 8.5 y. The median time to onset of efficacy was significantly shorter for all desvenlafaxine groups (50 mg, 12 d [*P*=0.02]; 100 mg, 9 d [*P*<0.0001]; 150 mg, 8 d [*P*<0.0001]; 200 mg, 6 d [*P*<0.0001]) than for placebo (29 d). Similar findings were obtained when measuring median time to onset of efficacy from the end of titration to 100 mg comparing desvenlafaxine 100 mg (7 d) versus placebo (29 d, *P*<0.0001).

Conclusion: Onset of efficacy is an important factor when considering treatment strategies for patients with VMS. The rapid onset of efficacy observed with the hormone-free desvenlafaxine and its overall efficacy support its use for the treatment of moderate to severe VMS in postmenopausal women.

1. Notelovitz M, Lenihan JP, McDermott M, Kerber IJ, Nanavati N, Arce J. Initial 17beta-estradiol dose for treating vasomotor symptoms. *Obstet Gynecol.* 2000;95(5):726-731.
2. Speroff L, Gass M, Constantine G, Olivier S; Study 315 Investigators. Efficacy and tolerability of desvenlafaxine succinate treatment for menopausal vasomotor symptoms: a randomized controlled trial. *Obstet Gynecol.* 2008;111(1):77-87.
3. Archer DF, Dupont CM, Constantine GD, Pickar JH, Olivier S; Study 319 Investigators. Desvenlafaxine for the treatment of vasomotor symptoms associated with menopause: a double-blind, randomized, placebo-controlled trial of efficacy and safety. *Am J Obstet Gynecol.* 2009;200(3):238.e1-238.e10.
4. Archer DF, Seidman L, Constantine GD, Pickar JH, Olivier S. A double-blind, randomly assigned, placebo controlled study of desvenlafaxine efficacy and safety for the treatment of vasomotor symptoms associated with menopause. *Am J Obstet Gynecol.* 2009 Feb;200(2):172.e1-10.

Sources of Research Support: Wyeth, which was acquired by Pfizer Inc in October 2009.

Nothing to Disclose: CJG-P, R-FC, WB

Pub # P1-311

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title Post-Discontinuation Patterns of Hot Flushes and Night Sweats after a 12-Week, Randomized, Masked, Placebo-Controlled Trial of Oral Micronized Progesterone in Healthy Menopausal Women 1-10 Years after Final Menstrual Flow

Author String JC Prior, CL Hitchcock
University of British Columbia, Vancouver, Canada

Body Hormone therapy (HT) is recommended for treatment of hot flush/night sweat (VMS). At least 25% of women cannot successfully discontinue due to rebound higher VMS with discontinuation (D/C) (1). Given that oral micronized progesterone (OMP) is effective VMS treatment (2) we hypothesized it caused no VMS rebound with D/C.

Trial Design: 4 wk post-therapy open-label D/C following 4-wk run-in, and 12-wk experimental.

Methods: Randomized, masked, placebo-controlled trial of OMP (2003-2009). Healthy, non-obese, non-smoking VMS treatment-seeking community women were eligible if no HT for 6 mo. Women recorded VMS frequency (#) and intensity (0-4) in a Daily Menopause Diary; VMS Score is the sum of day and night # X intensity. In 2006 women were asked to continue the Diary for 4-wk after D/C.

Interventions: Progesterone (300 mg Prometrium[reg]) or identical placebo at h.s. daily.

Outcome: VMS # and VMS Score weekly during D/C.

Randomization and masking: Computer-generated randomization was created, administered and maintained by an independent pharmacy; masking for subjects and researchers through data cleaning prior to analysis. At OMP/placebo D/C pharmacists disclosed therapy to participants who asked.

Results: Of 56 women randomized in the D/C protocol, 49 completed therapy. Analysis included 34 women (OMP n=17, placebo n=17) with Diary data in the 4th week of discontinuation. Women were aged 56.4±3.9 (mean±SD), 4.2 y (Q1,Q3: 2.2, 6.0) from last flow, BMI 24.7±2.7, waist circ. 77.8±6.8 cm. Most (89%) identified as white. Run-in daily VMS Score was 20.4±11.5 and # 7.8±3.3. Women completing D/C data were older (56.4 vs 53.4) but similar in weight, initial VMS Score and # to those who did not.

During the 4th week after OMP D/C both daily VMS # (6.5±3.8) and VMS Score (17.9±14.9) had increased from the last week on OMP (4.2±3.4 and 10.2±12.4), but remained below run-in (8.4±2.8 and 23.6±10.3). The 95% CI of differences for VMS# and VMS Score after 4-wk OMP D/C vs. last OMP were: (-3.3,-1.2) and (-11.5,-4.0), and for run-in vs. D/C: (0.8,3.0), (1.3,9.9).

Response to placebo was less than to OMP, and VMS # and VMS Score at wk-4 placebo D/C were not statistically different from the final therapy week or run-in (p>0.1 in all cases).

Conclusion: Women randomized to progesterone tended to return to pre-therapy VMS following discontinuation but remained lower than run-in during the 4th week of D/C.

Trial Registry: www.clinicaltrials.gov NCT00152438.

(1) Grady et al, Obs Gynec 2003, 102:1233.

(2) Prior et al, Endocrine Society Abstract, 2010

Sources of Research Support: Private individual donations to the Centre for Menstrual Cycle and Ovulation Research (CeMCOR). Active drug and placebo provided by Schering (Canada) and by Besins Healthcare.

Nothing to Disclose: JCP, CLH

Pub # P1-312
Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)
Title Changes in Cardiovascular Markers with Progesterone Therapy for Vasomotor Symptoms: A 12-Week Randomized, Masked, Placebo-Controlled Trial
Author String C Hitchcock, J Prior
University of British Columbia, Vancouver, Canada

Body Cardiovascular (CV) effects of oral micronized progesterone (OMP) vs placebo in a controlled trial are not available and are important given recent trial results (1-2). These CV measures are 2[deg] outcomes of a RCT of OMP for menopausal hot flushes (VMS). In a previous RCT we showed that medroxyprogesterone but not equine estrogen decreases inflammation and does not increase triglycerides (3). Both estradiol and progesterone improve endothelial function (4) and OMP vs placebo tended to improvement (5).
Objective: To compare CV factors on OMP vs placebo in women without preexisting CV risks.
Trial Design: 4-wk baseline then 12-wk experimental.
Methods: Randomized, masked, placebo-controlled trial of OMP at an academic center (2003-2009). Community women with VMS enrolled if within 1-10 y of LMP, no hormone therapy for 6 months, non-smoking, non-obese, no diabetes, hypertension, or heart disease, and were excluded for abnormal ECG, fasting lipids or glucose. Statistical analysis was by ANCOVA with baseline as covariate.
Interventions: OMP (300 mg at hs) or placebo daily.
Outcomes: Weight, waist circumference, blood pressure, serum lipids, inflammatory markers and D-dimer.
Randomization and masking: Computer-generated randomization was dispensed by an independent pharmacist. Participants and researchers were blinded.
Results: 133 women received OMP (n=75) or placebo (n=58). Women were aged 55.0±4.4 (mean±SD), 4.3±2.6 y from last flow, body mass index (BMI) 24.7±2.8, waist circ. 78.4±6.7 cm. Most (91%) identified as white and were not different by therapy assignment. There were no final differences (as 95% CI) between OMP (n=65) and placebo (n=47) in weight (-0.9,1.0 kg) waist circ. (-1.3,1.3 cm), systolic BP (-2.8,4.7) or heart rate (-2.9,2.4). There were no differences between OMP (n=63) and placebo (n=45) in total cholesterol (-0.30,0.04 mmol/L), LDL-C (-0.18,0.14 mmol/L) or triglycerides (-0.10,0.20 mmol/L). However, HDL-C was slightly decreased on OMP (-0.21,-0.07 mmol/L). Inflammation markers were also not different on OMP (n=33) vs placebo (n=25): C-reactive protein (-0.7,0.5 mg/L) and albumin (-1.7,0.6 g/L), nor did D-Dimer differ (-7.3,113.2 [mu]g/L FEU) between OMP (n=24) and placebo (n=19).
Withdrawal and Adverse Events: No serious adverse events occurred.
Conclusion: Progesterone (OMP) showed good safety across a range of clinical and laboratory cardiovascular markers in healthy menopausal women with VMS.
Trial Registry: www.clinicaltrials.gov NCT00152438.

1 Writing Group for the Women's Health Initiative Investigators. JAMA 2002; 288:321.

2 Hulley S et al., JAMA 1998; 280:605.

3 Kalyan S et al., Pharmacotherapy 2010; 30:442.

4 Mather K et al., JCEM 2000; 85:4644.

5 Hitchcock CL et al., Endocrine Society Abstract 2010.

Sources of Research Support: Private individual donations. Active drug and placebo provided by Schering (Canada) and by Besins Healthcare.

Nothing to Disclose: CH, JP

Pub # P1-313

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title LibiGel^[reg] (Testosterone Gel) Safety Study Enters Fourth Year with a Continued Low Rate of Cardiovascular and Breast Cancer Events

Author String MC Snabes, S Berry, JG Zborowski, WB White
BioSante Pharmaceuticals, Inc, Lincolnshire, IL; Berry Consultants, College Station, TX; University of Connecticut, Farmington, CT

Body

Objective: LibiGel^[reg] (testosterone gel) is being developed for treatment of hypoactive sexual desire disorder (HSDD) in postmenopausal women. A key to obtaining FDA approval for this indication is the demonstration of long-term cardiovascular (CV) and breast safety. Our testosterone gel CV and breast safety study is entering its fourth year of blinded data collection. The safety data are overseen by an independent Data Monitoring Committee (DMC). Herein we report on progress in this ongoing study.

Design: This is a randomized, placebo-controlled, CV events-driven, adaptive design, multi-center study. Postmenopausal women with HSDD and at least 2 CV risk factors who are enrolled receive either 300 mcg/d testosterone gel daily or an identical placebo gel. The primary safety outcomes are the effect of treatment on the incidence of an adjudicated CV event composite (CV death, myocardial infarction, stroke, hospitalized angina, revascularization, or venous thrombo-embolic events) and on the rate of breast cancer. Upon every review of unblinded safety data, the DMC can recommend continuing the study unchanged as per protocol, altering the study protocol or recommending that the study not continue.

Results: The study remains blinded to all except the DMC. To date over 2,822 post-menopausal women have been enrolled at a mean age of 59.0 years. Those enrolled have a mean duration of participation of 11.9 months. More than 87% of subjects continue in follow up. The overall rate of adjudicated CV events is 0.50% and the breast cancer rate is 0.29%. The DMC has completed four reviews of all unblinded safety data to date and each time the DMC recommended that the study continue as per protocol, without change.

Conclusions: This is the largest and longest controlled study of testosterone treatment in women. Although the study remains blinded to all except the DMC, the low CV event rate and breast cancer rate are reassuring particularly in women at the higher end of the CV risk continuum for the intended treatment population. The DMC recommendations to continue the study unchanged suggests there is no significant negative treatment effect of testosterone on the key safety outcomes. Assuming continued positive safety outcomes, demonstration of efficacy in the Phase III efficacy clinical trials, and a successful regulatory review of the LibiGel new drug application (NDA), LibiGel could be the first FDA approved therapeutic treatment of HSDD in postmenopausal women.

Disclosures: MCS: Employee, BioSante Pharmaceuticals, Inc. SB: Collaborator, BioSante Pharmaceuticals, Inc. JGZ: Collaborator, BioSante Pharmaceuticals, Inc. WBW: Collaborator, BioSante Pharmaceuticals, Inc.

Pub # P1-314
Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)
Title Testosterone Improves Verbal Learning and Memory in Postmenopausal Women: Results from a Pilot Study
Author String SL Davison, RJ Bell, M Gavrilescu, K Searle, P Maruff, A Gogos, SL Rossell, J Adams, P Robinson, GF Egan, SR Davis
Monash University, Melbourne, Australia; University of Melbourne, Parkville, Australia; CogState Ltd, Melbourne, Australia; University of Melbourne, Parkville, Australia; Mental Health Research Institute of Victoria, Parkville, Australia; Monash University, Prahran, Australia; Swinburne University, Hawthorn, Australia

Body **Background:** Testosterone levels decline in women from their early adult years, reaching a nadir in the mid 60s, after which time dementia incidence rises. There is evidence that testosterone exerts neuroprotective effects, however whether testosterone therapy can improve cognitive performance or prevent cognitive decline in women is unknown.
Objective: To explore the effects of testosterone on cognitive performance in healthy postmenopausal women.
Design: Open-label pilot study.
Setting: University research centre
Patients: Nine postmenopausal women on non-oral hormone replacement therapy, aged 47 to 60 years. A control group of 30 women provided normative data for comparison.
Intervention: Transdermal testosterone spray for 26 weeks.
Measurements: Scores from a computerized cognitive test battery, 'CogState', performed pre- and post treatment, at 0 and 26 weeks.
Results: There were no differences between treatment / normative groups in any parameter at baseline. At week 26 scores for the International Shopping list task including delayed recall (verbal learning and memory) and the Continuous Paired Associate Learning task (visual learning and memory) were significantly higher in the treatment group as compared to the normative group ($p < 0.05$). Significant improvements from baseline were observed for the International Shopping List delayed recall (verbal learning and memory) and Groton Maze recall tasks (visual learning and memory) for the treatment group (both $p < 0.05$), after 26 weeks. There were no significant differences between baseline and week 26 in the normative group. In the regression analysis which modeled the score at week 26, and which included a bootstrapping approach, the beta coefficient for the treatment group was statistically significant when age and baseline score were taken into account for the International Shopping List task including delayed recall (both $p < 0.02$).
Conclusion: Testosterone improved cognitive performance in the domain of verbal learning and memory in a pilot study of healthy postmenopausal women and is worthy of further exploration in a randomized placebo controlled study.

Sources of Research Support: National Health and Medical Research Council of Australia Grant numbers 490938 and 465145; Vincent Fairfax Family Foundation Fellowship, Royal Australasian College of Physicians, Research and Education Foundation; and FemPharm Pty Ltd.

Disclosures: SLD: Principal Investigator, Acrux Australia, Warner Chilcott Pharmaceuticals. PM: Employee, CogState Pty Ltd. SRD: Study Investigator, Biosante, Warner Chilcott Pharmaceuticals. Nothing to Disclose: RJB, MG, KS, AG, SLR, JA, PR, GFE

Pub # P1-315

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title Effects of Estradiol Valerate/Dienogest Compared with Ethinyl Estradiol/Levonorgestrel on Libido

Author String RE Nappi, SR Davis, S Parke, U Mellinger, M Serrani
IRCCS Maugeri Foundation, University of Pavia, Pavia, Italy; School of Public Health, Monash University, Melbourne, Australia; Bayer Schering Pharma AG, Berlin, Germany

Body

Objective: Free testosterone reduction may contribute to loss of libido with combined oral contraceptive (COC) use. In clinical practice, women suffering from COC-acquired female sexual dysfunction (FSD) are usually switched to ethinyl estradiol (EE) pills containing levonorgestrel (LNG), an androgenic progestin. Estradiol valerate/dienogest (E₂V/DNG) leads to less pronounced decreases in total and free testosterone and dehydroepiandrosterone-sulfate, and a lesser increase in sex hormone binding globulin than a COC containing EE/LNG. This multi-center, double-blind, randomized study in Europe and Asia/Pacific evaluated the effects of E₂V/DNG and EE/LNG on libido in COC-acquired FSD.

Methods: Healthy female COC users (18-50 years; smokers 18-30 years) with COC-acquired FSD (female sexual function index [FSFI] sexual desire and sexual arousal component score [le]18) for [ge]3 months but [le]1 year, and willing to switch their COC, were randomized to E₂V/DNG (estrogen step-down and progestin step-up regimen) or EE (0.03 mg)/LNG (0.15 mg) (monophasic 21/7-day regimen) for 6 cycles. The primary efficacy outcome was change from baseline to cycle 6 in the sum of FSFI sexual desire and sexual arousal domain scores. Secondary efficacy outcomes included individual domains of FSFI, Female Sexual Distress Scale (FSDS-R) scores and evaluation of vaginal effects (pH, Vaginal Health Assessment [VHA] and Atrophy Symptom Questionnaire [ASQ]).

Results: 191 women completed the study. The mean increase in the FSFI desire and arousal domain scores was 5.90 (standard deviation [SD]: 5.45) with E₂V/DNG and 5.79 (SD: 6.17) with EE/LNG (last observation carried forward; p<0.0001 for both groups). The COCs were equally effective for improving the FSFI domain scores for desire, arousal, lubrication, orgasm and satisfaction, and for reducing pain during or following vaginal penetration. Both treatments also equally improved subjective distress associated with FSD (FSDS-R total score). Neither treatment changed vaginal pH or composition and appearance of the vagina (VHA sum score). The ASQ score increased with both treatments, indicating that subjects perceived an improvement in the status of the vagina.

Conclusion: E₂V/DNG showed no difference to EE/LNG in terms of libido in women with COC-acquired FSD. Both E₂V/DNG and EE/LNG improve COC-associated FSD.

Disclosures: REN: Consultant, Bayer Schering Pharma; Boehringer Ingelheim; Novo Nordisk; Proctor & Gamble; Merck & Co.; Schering Plough; Organon Laboratories. SRD: Study Investigator, Biosante; Warner Chilcott Pharmaceuticals. SP: Employee, Bayer Schering Pharma. UM: Employee, Bayer Schering Pharma. MS: Employee, Bayer Schering Pharma.

Pub # P1-316

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title Transient Acromegaly during Pregnancy Due to Excessive Placental Growth Hormone

Author String S Hegde, I Zamfirescu, C Avila, P Ogburn, M Charitou, M Bidlingmaier, HE Carlson
Stony Brook University, Stony Brook, NY; Stony Brook University, Stony Brook, NY; Ludwig-Maximilians University, Munich, Germany

Body

Introduction: Mild soft tissue enlargement is sometimes noted in late pregnancy, perhaps related to placental GH (pGH) production. We report a unique case in which excess pGH caused severe soft tissue enlargement with airway compromise, and was associated with hirsutism and marked hyperandrogenism. The soft tissue changes altered the management and course of pregnancy, and rapidly regressed after delivery.

Case: A 30-year old woman, 27 weeks pregnant, presented with non-pitting soft tissue enlargement of hands, feet and facial features and progressive difficulty with breathing at night. Her tongue was enlarged and she noted increased facial hair. Six years prior, she had similar changes during pregnancy that resolved after delivery. Her renal, thyroid and cardiac function were normal. Laboratory tests showed low serum hGH of 0.07 ng/mL (N<0.5), markedly elevated IGF-1 >1600 ng/mL (N=100-500), IGF-BP3 8140 ng/mL (N=2878-9000), IGF-BP1 12 ng/mL (N=50-250), serum GH-binding protein 573 pmol/L (N=686-2019), testosterone 627 ng/dL (N=14-76) and DHEA 2.03 ng/mL (N=1.3-7.8). Her ovaries were enlarged (10 cm) with multiple thin-walled cysts, consistent with hyperreactio luteinalis. A sleep study showed obstructive sleep apnea and Continuous Positive Airway Pressure (CPAP) was initiated. At 29 weeks, pGH was 54 ng/mL (N<25). At 31 weeks, she had continued enlargement of her extremities and shortness of breath, despite use of CPAP. Lab results showed low hGH of 0.18 ng/mL, pGH 79.3 ng/mL, IGF-1 2163 ng/mL, IGF-BP3 11,200 ng/mL, placental lactogen 10.6 mcg/mL (N<12.8), testosterone 1030 ng/dL, SHBG 83 nmol/L (N=30-135), hCG 134,000 mIU/mL (N<50,000). Pituitary MRI was normal. Due to progression of airway obstruction, she had a cesarean section at 32 weeks with delivery of an unvirilized 3275-gram girl. After delivery, soft tissue swelling decreased and breathing improved. 7 days later pGH was <0.05 ng/mL and IGF-1 was 145 ng/mL. 21 days post-partum, serum testosterone was 20 ng/dL. Ultrasound 2 months post-partum showed normal ovaries with no cysts.

Conclusion: We describe a new syndrome of gestational acromegaly due to abnormally high concentrations of pGH. In addition to soft tissue changes and airway obstruction, hirsutism and hyperandrogenism due to hyperreactio luteinalis was seen, possibly driven by elevated serum IGF-1 and hCG. Early cesarean section was needed to reverse this syndrome, which rapidly improved after delivery.

Nothing to Disclose: SH, IZ, CA, PO, MC, MB, HEC

Pub # P1-317

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title The Presence of FSH Receptor Polymorphism Asn680Ser Requires Increased Exogenous FSH for Oocyte Production during Ovarian Stimulation for *In Vitro* Fertilization

Author String AM Seungdamrong, PG McGovern, A Wojtczuk, LT Goldsmith
New Jersey Medical School - UMDNJ, Newark, NJ

Body
Background: An FSH receptor single nucleotide polymorphism (SNP), resulting in a change in amino acid 680 from asparagine to serine, alters FSH action. Thus, we hypothesize that this SNP alters the ovarian response to exogenous FSH administered during controlled ovarian stimulation (COS) for in-vitro fertilization (IVF) manifesting altered ovarian hormone and gamete production.
Methods: A prospective cohort study of all IVF patients under 35 years old seen between 10/2009 and 1/2011 was performed. Inclusion criteria included: 1) basal FSH < 10 mIU/ml, 2) basal estradiol < 70 pg/ml, and 3) ovarian antral follicle count \geq 10. Patients with polycystic ovarian syndrome or endometriosis were excluded. DNA was extracted from blood samples using the Qiagen Flexigene kit. Genotyping was performed with an ABI Taqman SNP assay (C_2676874_10). ANOVA and logistic regression were used for statistical analysis.
Results: 36 of 124 IVF patients during the study period met inclusion criteria. The genotype frequency was 0.28 (10/36) for the Asn/Asn genotype, 0.61 (22/36) for the Asn/Ser genotype, and 0.27 (4/36) for the Ser/Ser genotype. Mean patient age was not significantly different among the groups ($p=0.45$). Mean total doses of exogenous FSH were 2093 IU (Asn/Asn), 2259 IU (Asn/Ser), and 2291 IU (Ser/Ser) ($p = 0.89$). Mean maximum estradiol levels were 2153 pg/ml (Asn/Asn), 2078 pg/ml (Asn/Ser), and 1752 pg/ml (Ser/Ser) ($p = 0.61$). Pregnancy rates were 50% (Asn/Asn), 62% (Asn/Ser), and 50% (Ser/Ser) ($p = 0.68$). Mean numbers of oocytes retrieved per patient were 18 (Asn/Asn), 13 (Asn/Ser), 11 (Ser/Ser). In contrast, a significant negative correlation was found between the serine genotype and the number of oocytes retrieved per patient, $p = 0.045$. Importantly, a significant positive correlation was found between the serine genotype and the amount of exogenous FSH used per oocyte retrieved, $p = 0.027$.
Conclusions: The Asn680Ser SNP affects the amount of exogenous FSH needed to stimulate the ovary to produce oocytes during controlled ovarian stimulation for IVF. While exogenous FSH is titrated carefully to achieve a similar maximum amount of estradiol during each IVF cycle, the number of oocytes retrieved per unit of exogenous FSH decreases with increasing presence of the serine allele. This finding is likely to improve our ability to individualize patient care for improvement of fertility treatment.

Sources of Research Support: Ferring Pharmaceuticals Investigator Initiated Grants.

Nothing to Disclose: AMS, PGM, AW, LTG

Pub # P1-318

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title Severe Hyperandrogenism and Polycystic Ovary Syndrome (PCOS) in Acromegaly

Author String S-M Kim, H Nguyen, J Kim, N Blocher
Albert Einstein Medical Center, Philadelphia, PA; Albert Einstein Medical Center, Philadelphia, PA

Body Introduction: Mechanisms underlying PCOS and hyperandrogenism in women are incompletely known and likely multifactorial. The GH/IGF-1 axis is implicated (1), for example via over-stimulation of ovarian IGF-1 receptors. In support, octreotide has successfully treated some obese women with PCOS (2). Interestingly, PCOS occurs in a minority of women with acromegaly (3). We present a patient with acromegaly, PCOS, and Testosterone levels over five times the upper limit of normal that is resolving after successful removal of a somatotroph adenoma.

Case: A 26 yr old African American female presented with PCOS diagnosed at age 17 unresponsive to treatment. Since menarche at 12, she had slow, steady progression of irregular menses, oily skin, increased pigmentation and diffuse hirsutism. At presentation she shaved daily and had two menses in the prior year. She had carpal tunnel syndrome, osteoarthritis, and obstructive sleep apnea. She perspired excessively and had enlargement of her hands and feet. No family history of diabetes mellitus or PCOS. She was 5'9" tall with a BMI 34. Breasts and genitalia were normal, but her body was masculine with broad shoulders, diffuse acanthosis nigricans, and grade 3-4 hirsutism at all locations. Her facial features were coarse and her fingers wide.

At presentation, **Total Testosterone (TT) = 266 ng/dl** (nl 2- 45), free testosterone (fT) = 58.6 pg/ml (nl 0.2-5.0), SHBG = 16 nmol/L (nl 6-112), LH 1.9 mIU/mL. FSH, DHEA-S, androstenedione, 17-OHP, Prolactin, ACTH, thyroxine, cortisol, & HbA1c were normal.

IGF-1 = 1061 ng/ml (nl for age 88-374). On ultrasound, ovaries were large with multiple cysts. MRI showed a 1.4 cm round, sellar mass.

One month after radiographically complete adenoma resection, TT = 96 ng/dl while IGF-1 = 507 ng/ml. (It can take three months for IGF-1 to normalize.) She had menstruated and joint discomfort was better.

Discussion: Hyperandrogenism to this degree is not common in PCOS or acromegaly. The pathogenic role of IGF-1 is supported by type A insulin resistance syndrome, where insulin may cross-bind to IGF-1 receptors to induce severely elevated testosterone like our patient along with PCOS, acanthosis nigricans, and sometimes acromegalic features or "pseudoacromelagy".

Conclusion: Our case supports the role of the GH/IGF-1 axis in the pathogenesis of PCOS. The remaining question is why elevated IGF-1 or insulin causes hyperandrogenism to varying degrees in some women and not others.

(1) Poretsky et al. Endo Rev 1999; 20:535-582
(2) Gambineri et al. JCEM 2005; 90:3854-3862
(3) Grynberg et al. JCEM 2010; 95:4518-4525

Nothing to Disclose: S-MK, HN, JK, NB

Pub # P1-319

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title A Case of Recurrent Virilization during Pregnancy

Author String N Couture, HB Lavoie
Centre Hospitalier de l'Université de Montréal, Montréal, Canada

Body

Case report

A healthy 29-year-old African woman, gravida 2, para 2, is referred to us by her family doctor for clitoromegaly with high DHEA 58.8 (N=2.7-36.3 nmol/L), 11 months after giving birth to her second baby boy.

She had her menarche at 16 years old followed by regular menstrual cycles. During her first pregnancy, clitoromegaly as well as increased libido, facial hirsutism, acne and deepening of her voice were noted. At caesarean section, the gynecologist mentioned adnexal anomalies compatible with a hyperreactio luteinalis. All symptoms recurred during her second pregnancy and at delivery, both ovaries were enlarged (5x4 cm each).

Her symptoms improved after each delivery except for the deepening of her voice and she was able to breastfeed immediately after giving birth. A pelvic ultrasound performed 4 months after her second delivery was normal. Hormonal testing was also normal postpartum: total testosterone 1.6 (N<2.9 nmol/L), free testosterone <6.0 (N<11.0 pmol/L), androstenedione 4.4 (N=2.8-13.9 nmol/L), DHEAS 5.8 (N=0.8-11.3 [mu]mol/L), SHBG 28 (N=22-104 nmol/L), 17-OHP 2.8 nmol/L, PRL 18.8 (N=3-29 [mu]g/L), LH 4.5 UI/L and FSH 4.9 UI/L. The patient is now worried about a third pregnancy!

Discussion

The two major causes of hyperandrogenism during pregnancy are pregnancy luteoma and hyperreactio luteinalis. Both are benign hyperplastic lesions.

Pregnancy luteomas are usually solid lesions of hyperplastic theca or stromal cells. They can be unilateral or bilateral and most commonly occur in black multiparous women, particularly of Afro-Caribbean descent and in women with PCOS. Virilization is encountered in up to 50% of women and there is a 50% risk of masculinization of a female fetus.

Hyperreactio luteinalis is most frequently bilateral and represents enlarged ovaries with multiple theca lutein cysts. It most commonly occurs in white primiparous and is more frequently seen in conditions associated with a high hCG value, such as molar pregnancies and multiple gestations. Virilization is seen in 25% of women. No fetal virilization is reported.

Both conditions spontaneously regress after delivery, but they can rarely recur in subsequent pregnancies.

Conclusion

The clinical history of this woman is compatible with recurrent hyperreactio luteinalis even if we don't have hormonal testing performed during her pregnancies. If she becomes pregnant again, we will measure her androgen levels.

Nothing to Disclose: NC, HBL

Pub # P1-320

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title A 14-Year-Old Girl with MURCS Association and Hyperandrogenism

Author String ZF Shoar, CE Anderson, R Prasad, F De Luca, EA Suarez
St Christopher's Hospital for Children, Drexel University College of Medicine, Philadelphia, PA; St Christopher's Hospital for Children, Drexel University College of Medicine, Philadelphia, PA; St Christopher's Hospital for Children, Drexel University College of Medicine, Philadelphia, PA

Body Background: MURCS (Mullerian aplasia, Renal anomalies, and Cervicothoracic Somite dysplasia) association is a rare finding characterized by genito-urinary and skeletal defects. Here we report an adolescent girl with the cardinal features of MURCS association and clinical findings of hyperandrogenism.
Clinical Case: A 14-1/12 year old female presented with primary amenorrhea. Breast and pubic hair development occurred at age 10 years. Her past medical history was significant for encephalocele (diagnosed prenatally), XX genotype (diagnosed prenatally), cervical cord syrinx, Klippel-Feil syndrome, VP shunt placement, and left renal agenesis. She had global developmental delay that required occupational, physical, and speech therapy.
Her family history was negative for amenorrhea or congenital malformations.
On physical exam, her height was 146.7cm (<3rd percentile) and her weight 82 kg (> 97th percentile). While the left breast was consistent with Tanner stage 5, the right breast was much smaller (prominent areola with small breast bud). Pubic hair development was consistent with Tanner stage 5 and no vaginal opening was observed. Terminal hair was noted on her chin, sideburns, upper lip, and abdomen. The rest of the physical exam was notable for the presence of facial asymmetry, short neck with limited range of motion, clinodactyly and one crease on her left palm.
Laboratory work up revealed elevated testosterone (67 ng/dL), borderline-high 17-OHP (158 ng/dL), with normal LH (2.89 mIU/mL), FSH (3 mIU/mL), DHEAS (243 mcg/dL), androstenedione (152 ng/dL), and estradiol (83 pg/mL). MRI showed absent uterus and upper one-third of vagina, a blind vaginal pouch, left intra-abdominal and right intra-inguinal gonads, absent left kidney, and spondylolisthesis of L5 on S1. Laparoscopy identified the ectopic gonads as ovaries; it also identified a rudimentary right fallopian tube.
Conclusion: To our knowledge, this is the first patient reported with MURCS association and hyperandrogenism. Previously, an adult woman with mullerian-duct regression (but without skeletal defects) and virilization was reported with a loss-of-function mutation of the Wnt4 gene, which encodes for a secreted molecule essential for normal ovarian and uterine development. Genetic investigation of Wnt4 and other genes involved in the differentiation of mullerian duct, kidney, and skeleton may help clarify the underlying mechanisms of the novel association described in our patient.

Nothing to Disclose: ZFS, CEA, RP, FDL, EAS

Pub # P1-321
Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)
Title Virilizing Dermoid Cyst in a Postmenopausal Woman
Author String S Subbarayan, L Olansky
Cleveland Clinic, Cleveland, OH

Body **Background:** Androgen secreting ovarian tumors are rare, with virilizing dermoid cysts being rarer still. Only 8 such cases are reported in the literature.

Case: A 58-year old female patient was evaluated for hirsutism and abdominal pain. She had excess facial hair since age 20 which worsened in the past year to the point that she had to shave daily. She reported increased libido and frontal balding in the past year. Menses had been irregular since menarche till her menopause 5 years ago. She had one child who was conceived naturally with no subsequent pregnancies despite absent contraception. She denied clitoromegaly, muscle weakness, acne and stretch marks. Past medical history included poorly controlled diabetes on insulin treatment, PCOS, hypertension and stroke. Exam revealed male habitus, frontal baldness, deep voice, hirsutism and generalized obesity. She did not look overtly Cushingoid. Patient declined a genital exam. Testosterone was 200 ng/dl (20-70), DHEA-S 172.3 ng/dl(<10-152), DHEA 2.5 ng/ml(<6), Androstenedione 1.7 ng/ml (0.5-2.7) and FSH 50.5 mU/ml. 24 hour urine free cortisol was normal. A left ovarian mass measuring 20 x 19 mm was detected on CT abdomen/pelvis and transvaginal ultrasound. Adrenals were normal. Patient underwent hysterectomy with bilateral salpingo-oophorectomy. Pathology showed benign dermoid cyst with a zone of luteinized cells at the periphery. Testosterone decreased to 61 ng/dl postoperatively.

Conclusion:

Dermoid cystic teratoma is a germ cell tumor and does not secrete androgens in and of itself. It is speculated that slow growing tumors cause pressure on the stromal cells inducing luteinization. The exact stimulus remains unclear. There may be an interaction of several factors. Specific binding sites for insulin and IGF-1 in the ovarian stroma in patients with PCOS and hyperthecosis have been demonstrated¹. It is proposed that insulin resistance and hyperinsulinemia may act synergistically at the IGF-1 receptor level, with possible contribution by the elevated gonadotropins in the postmenopausal state, leading to androgen production by stromal cells. 3 cases with virilizing mature cystic teratomas in postmenopausal, obese, diabetic women, similar to our patient have been described¹. Of note, our patient had a history of PCOS. There are reports of androgen receptors in ovarian tumors, raising speculation that increased intraovarian androgen levels have a role in the genesis of androgen secreting tumors².

(1) Nagamani .Am J Obstet Gynecol. 1990 Dec;163(6 Pt 1):1992-7

(2) Aiman J. Clin Obstet Gynecol. 1991 Dec;34(4):835-47

Nothing to Disclose: SS, LO

Pub # P1-322

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title Normal Pseudoautosomal Region 1 Gene Dosage in a 46,XX Disorder of Sex Development (DSD) Ovotesticular SRY-Negative Patient with Rheumatologic Overlap Syndrome: Systemic Lupus Erythematosus and Systemic Sclerosis

Author String MGM Rocha, MFA Funari, MTC Caleiro, MY Nishi, RB Silva, BB Mendonca, EMF Costa, S Domenice Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Body The gene dosage of X chromosome has been suggested to play an important role in the pathogenesis of autoimmune diseases (AID). The concurrence of Klinefelter's syndrome (KS) with AID such as rheumatoid arthritis, systemic lupus erythematosus (SLE), systemic sclerosis (SS) and mixed connective tissue disease (MCTD) has been frequently reported and reinforces this proposal. Chagnon et al. (1) described a 46,XX DSD testicular patient with a t(X;Y)(p22.33;p11.2) and SLE. This patient presented SRY and three copies of the pseudoautosomal region 1 (PAR1) genes similar to that observed in KS patients. Objective- To evaluate the gene copy numbers of the PAR1 in a 46,XX DSD ovotesticular SRY-negative patient with a Rheumatologic Overlap Syndrome (ROS; Systemic Lupus Erythematosus and Systemic Sclerosis). Case- The patient had ambiguous genitalia (microphallus, penoscrotal hypospadias, bifid scrotum) and inguinal gonads. The karyotype was 46,XX. At age 1.75 yrs, he was submitted to exploratory laparotomy and bilateral gonadectomy was performed. The diagnosis of 46,XX DSD ovotesticular was confirmed by histological findings of bilateral ovotestes. At the age of 11 yrs, testosterone replacement was initiated and male secondary sexual characteristics developed. The manifestation of rheumatologic disease began at 14 yrs, with an initial diagnosis of MCTD, according to Kasukawa's criteria. Five years later the auto-antibodies profile changed, leading to a new diagnosis of ROS-SLE and SS, according to ACR criteria. At 17 yrs Hashimoto's thyroiditis was diagnosed. Methods- Genomic DNA was extracted from peripheral blood leukocytes. The copies dosage of PAR1 genes were performed by multiplex ligation probe amplification (MLPA) with Kit P018D1 SHOX. Results- The MLPA did not identify any copy number variation in the PAR1. Discussion- The risk of SLE in KS is predicted to be approximately 14-fold higher than in men. Low testosterone levels found in KS might be a predisposing factor for the development of AID. The influence of sex chromosome genes has also been suggested as a trigger factor. Recently, excess of PAR1 genes dosage was suggested to be associated with the high incidence of AID in KS. In this 46,XX DSD ovotesticular patient with ROS the copy number of the PAR1 genes were not in excess of dosage. Conclusion- This result suggests that excess of PAR1 genes dosage is not involved in the development of ROS in this 46,XX DSD ovotesticular SRY-negative patient.

(1) Chagnon P et al., Arthritis and Rheumatism 2006; 54:1270

Nothing to Disclose: MGMR, MFAF, MTCC, MYN, RBS, BBM, EMFC, SD

Pub # P1-323

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title Aromatase Deficiency in a 17-Year-Old Brazilian Girl: Case Report and Description of a Novel Mutation

Author String S Nesi-Franca, R Marino, R Marques-Pereira, J La Pastina, N Perez Garrido, N Saraco, R Sandrini, MA Rivarola, A Belgorosky, L De Lacerda
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Body Aromatase deficiency is a rare autosomal recessive disorder in which affected patients cannot synthesize estrogens. Fetus lacking aromatase activity are not able to convert DHEA-S produced by the placenta to estrogens; DHEA-S is converted to testosterone resulting in virilization of both fetus and mother. We report a girl born of non-consanguineous parents, seen at Pediatric Endocrinology Unit at age 4 due to 46,XX DSD, who was treated from birth to 3 y with glucocorticoid and fludrocortisone. Laboratory evaluation discarded congenital adrenal hyperplasia. She was submitted to external genitalia plastic correction and laparoscopy showed normal Müller structures. At 13 y Tanner stage was B1P4, acne and mild hirsutism were present; bone age was 9.5 y, LH and FSH 25.3 and 14.1 mIU/mL, respectively, and estradiol 27.7 pg/mL. Pelvic ultrasound revealed small uterus and presence of cysts in both ovaries. Puberty was induced with conjugated estrogens and subsequently progestogens, with regular menses. At age 17 y she measured 157.5 cm (height Z score -1.0 midparental height Z score -2.2) and no growth spurt had been observed; bone age was 13 y; pelvic ultrasound showed multicystic, enlarged ovaries (7 and 10 cc, right and left, respectively). Aromatase deficiency was suspected. Genomic DNA analysis of the P450arom gene of the patient, parents and three siblings (2 girls and 1 boy) showed an intronic homozygote mutation (IVS9+5G>A) in the patient and heterozygosity for the same mutation in parents and siblings. By using the splicing prediction program (Splice View) it was confirmed that the splicing donor site disappears in the presence of that mutation. Differently to what has been reported, the mother did not show any sign of virilization during pregnancy. Further studies are necessary to confirm the functional consequences of the above mentioned mutation.

- (1) Shozu M et al., J Clin Endocrinol Metab, 1991; 72:560.
- (2) Belgorosky A et al., Horm Res 2010;72:321.

Nothing to Disclose: SN-F, RM, RM-P, JL, NPG, NS, RS, MAR, AB, LDL

Pub # P1-324

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title Gonadal Peptide Hormones in P450c17-Deficient Patients

Author String A Essam, M Taisseer, M Shaaban, D Klingmueller
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Body

Deficiency of Cytochrome P450c17, a member of the microsomal steroidogenic enzymes, is a very rare autosomal recessive form of congenital adrenal hyperplasia caused by CYP 17A1 gene mutation. The consequence is an impaired synthesis of both testosterone and cortisol and thus a rise in mineralocorticoid production. Androgens are essential for the spermatogenesis and Sertoli cells. To figure out the Sertoli cell function in affected patients we measured gonadal peptide hormones (inhibin B and Anti-Müllerian hormone, AMH).

We report on two phenotypic female patients from Kuwait characterized by primary amenorrhea and sexual infantilism with low cortisol and low sex hormones. The patients are siblings born by consanguineous parents (first-degree cousins). They were raised as girls and at the age of 15 and 22 years they were presented to the physician because of primary amenorrhea and lack of pubertal development. They had prepubertal female external genitalia but no uterus. The external genitalia displayed the female phenotype with a blind ending vagina. The karyotype was 46, XY. The elder one had high blood pressure and hypokalemia. The multiplex ligation-dependent probe amplification - method of this patient revealed a deletion of exon 1 to 6 in the CYP 17A1 gene.

The hormone analysis showed the typical pattern of accumulated DOC, corticosterone and progesterone as well as decreased synthesis of 17OH-progesterone, DHEAS, cortisol, testosterone and estradiol. The increased gonadotropins are typical for the primary gonadal failure. Striking was the subnormal inhibin B in both patients (2 and 10 pg/ml; normal range 60-300 pg/ml). AMH (2.8 and 5.2 ng/ml) was in the normal range for healthy men (1.5-4.3 ng/ml), but subnormal for prepubertal patients (10-130 ng/ml).

The enzyme deficiency is responsible for a disturbed sex hormone synthesis and secretion; and the low testosterone may affect the production of the gonadal peptide hormones (inhibin B and AMH) in Sertoli cells. AMH production remains high throughout childhood in males but declines to low levels during puberty and adult life. In our patients AMH was normal for adult males, but subnormal for prepubertal male patients. Nevertheless, the absence of the uterus suggests the presence of the AMH during the fetal period, because AMH prevents the development of the müllerian ducts into the uterus. Our findings confirm that testosterone is indispensable for the Sertoli cell function.

Nothing to Disclose: AE, MT, MS, DK

Pub # P1-325

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title Preserving Bone Health in a Young Female with Panhypopituitarism Who Developed Two Estrogen-Dependent Tumors on Oral Contraceptives

Author String CA Tibble, CA Stuenkel
University of California San Diego, San Diego, CA

Body

Background: The standard of care of young women with panhypopituitarism includes estrogen and progesterone replacement typically in the form of oral contraceptives. In women who develop complications from OCs, options for estrogen replacement and bone preservation have not been definitively addressed in the literature.

Clinical Case: A 35 year old female was diagnosed with pinealoma at age 12, shortly after menarche. The tumor was successfully treated with chemotherapy and cranial irradiation. She developed central DI, adrenal insufficiency, and hypothyroidism, which were treated. She continued to menstruate spontaneously until OCs were started at age 15 for menorrhagia. At age 28, a cranial meningioma was removed. She continued on OCs. She reports taking alendronate in the past; a DEXA in 2009 showed a T-score of -1.8. At age 35, she presented to the emergency room with acute abdominal pain. Abdominal CT scan demonstrated a 7cm hemorrhagic hepatic tumor. The tumor, a hepatocellular adenoma, required resection to control hemorrhage. Postoperatively, we confront the dilemma of how to preserve bone health in a young woman with pituitary dysfunction who developed two estrogen-dependent tumors while on OCs. After a thorough literature search we conclude that OCs should not be reinstated given the risk for recurrent hepatocellular adenomas. In additional discussions with menopause experts, we determine the safest course of action may be to await spontaneous return of menses, though anecdotal reports suggest that endogenous estrogen might also promote tumor recurrence. If amenorrhea persists for > 1 year and central hypogonadism is confirmed, a very low dose of transdermal estrogen is a strategy to cautiously consider, thus avoiding hepatic first-pass effect while providing systemic estrogen benefits. Concurrently, we will monitor closely for other osteoporosis risk factors including deficiencies of vitamin D, calcium and GH, and over-replacement of thyroxine and glucocorticoids.

Conclusion: This patient raises challenging questions on how to preserve bone health in a young panhypopituitary woman who may not have achieved peak bone mass, and has a history of hepatocellular adenoma and meningioma. A trial of low dose transdermal estrogen should only follow extensive risk-benefit discussions with the patient, with ongoing surveillance for potential complications. Nonhormonal agents for bone preservation could be considered in the future.

Nothing to Disclose: CAT, CAS

Pub # P1-326

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title Inadvertent Transfer of Cutaneous Estrogens from Women to Cats and Dogs

Author String CA Stuenkel, PD Pion, E Lau
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Body Background: In the wake of the announcement of the results of the Women's Health Initiative in 2002, use of transdermal estrogen therapy, both FDA approved and compounded estrogen creams, increased. While FDA procedure for cutaneous therapies (gels, sprays, emulsions) includes some pre-approval evaluation of transferability between adults, one case of human to pet transfer of estrogen cream has been published (1), and the FDA is reviewing several other reports. Awareness by the human medical community that small animals are vulnerable to inadvertent exposure to cutaneous estrogen used by their owners is lacking.

Clinical Cases: The Veterinary Information Network, a subscription online resource for veterinarians, compiled nearly 50 reports of cats and dogs presenting with signs and symptoms of estrogen excess, including labial enlargement in females, nipple enlargement in both genders, and occasionally, alopecia. Signs of estrus were observed in neutered or sexually immature pets. Evaluation included questioning owners about hormone exposure, and in some cases, determination of elevated serum estradiol levels. Some previously neutered patients were subjected to surgical exploration for retained ovaries. After repeated questioning, the attending veterinarians realized their patients had been exposed to estrogen applied to their owners' hands, arms, legs, or abdomens. The majority of cases involve compounded estrogen creams; the remainder used an FDA approved estrogen spray, Evamist. Owners described dogs licking their hands and arms where estrogen had been applied or cuddling with their pets which would then lick their fur. In some instances, pets were reported to ingest or play with disposed estrogen products. When exposure was reduced by changing the site of application, restricting pet access to the products, or switching to another mode of estrogen therapy, signs and symptoms of estrogen excess in the affected pets abated, usually over several months.

Clinical Lessons: Caution women at the time cutaneous estrogen prescriptions are written that inadvertent exposure to pets can occur. Appropriate hygiene measures should be followed to avoid transfer: carefully wash hands after application, allow time for proper drying prior to contact, consider the use of clothing to cover the area of application. Do not allow pets to lick skin where estrogen has been applied. If pets develop signs, inform their veterinarian of possible estrogen exposure.

(1) Schwarze RA, Threlfall WR. Theriogenology question of the month. JAVMA 2008;233:235237.

Nothing to Disclose: CAS, PDP, EL

Pub # P1-327

Session Information POSTER SESSION: CLINICAL - Female Reproduction: Clinical Trials & Case Reports (1:30 PM-3:30 PM)

Title Effect of Raloxifene on Human Neurocysticercosis

Author String AS Sacerdote, O Salamon, G Bahtiyar
Woodhull Medical Center, Brooklyn, NY; Woodhull Medical Center, Brooklyn, NY; SUNY Downstate Medical Center, Brooklyn, NY; NYU School of Medicine, New York, NY; NYU School of Medicine, New York, NY; St George University, School of Medicine, St George, Grenada

Body We've previously reported a patient whose PCOS and increased calcitriol level were associated with endocrin disrupter effects of neurocysticercosis. The patient, a 34 year old woman from Mexico, refused standard therapy for neurocysticercosis. Based upon data reported by Larralde et al on the effect of treatment with tamoxifen in murine cysticercosis, the patient, although premenopausal, was offered treatment with raloxifene after giving her informed consent, having a negative pregnancy test, and agreeing not to become pregnant while on treatment.

The patient began raloxifene HCl 60 mg orally daily on 1-21-10. She returned to clinic on 3-17-10 reporting that she'd become pregnant and was referred to Family Planning for termination, which was performed on 4-14-10. Raloxifene was stopped. Repeat MRI on 4-26-10 showed diminution in size, shrinkage, and loss of viability in a number of the cysts. Total lesions fell from 37 to 33, 10 lesions got smaller, 5 resolved, 18 were unchanged, 4 got larger, and only 1 new lesion developed. On 5-5-10 she started a 2 week course of standard treatment with albendazole and dexamethasone.

We conclude that alteration of the hormonal milieu may be an effective therapy in human neurocysticercosis, signaling a possible paradigm shift in the treatment of this disorder. The pregnancy, which occurred while taking raloxifene, though unplanned and unfortunate, provides further support of concept that the neurocysticercosis caused the patient to develop PCOS, as the selective modification of the hyperestrogenic milieu resulted in loss of viability of some cysts and exerted a permissive effect on conception. Patients of reproductive age should exert extreme caution not to conceive while on selective estrogen receptor modulators.

Vargas-Villavicencio JA, Larralde C, De Leon-Nava MA, Escobedo G, Morales_Montor J. Tamoxifen treatment induces protection in murine cysticercosis. *J Parasitol* 2007; 93:1512-7.

Nothing to Disclose: ASS, OS, GB

Pub # P1-328

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title The Effect of Gonadotropin Withdrawal and Stimulation with Human Chorionic Gonadotropin on Intratesticular Androstenedione and DHEA in Normal Men

Author String MY Roth, ST Page, K Lin, BD Anawalt, AM Matsumoto, B Marck, WJ Bremner, JK Amory
University of Washington, Seattle, WA; University of Washington, Seattle, WA; University of Washington, Seattle, WA; Veterans Affairs Puget Sound Health Care System, Seattle, WA

Body

Introduction: Concentrations of intratesticular (IT) testosterone are known to be 100-200 times those of serum testosterone (T); however the IT concentrations of testosterone's precursors, their testicular to serum gradients gonadotropin dependence and response to stimulation with human chorionic gonadotropin (hCG) have not been studied in detail. We hypothesized that serum and IT-androstenedione (IT-ADD) and IT-dehydroepiandrosterone (IT-DHEA) would be significantly suppressed by the administration of a gonadotropin-releasing hormone (GnRH) antagonist, and increased when stimulated by hCG, without a similar suppression of serum DHEA.

Methods: We suppressed gonadotropins in 23 normal men with the GnRH antagonist acyline and randomly assigned them to one of four doses of hCG - 0 IU, 15 IU, 60 IU or 125 IU subcutaneously every other day for 10 days. Blood and IT fluid for the measurement of serum and IT hormones were obtained at baseline and after 10 days of treatment using a testicular fine-needle aspiration technique under local anesthesia. Hormones were measured using liquid chromatography-mass spectrometry.

Results: Baseline IT-ADD [median (25th, 75th percentile)] was 629 (308, 860) nmol/L and IT-DHEA was 564 (411, 879) nmol/L, which were 175 and 27 times higher than their respective serum concentrations. IT-ADD and IT-DHEA were suppressed by 98% and 82% respectively by acyline and significantly increased with hCG administration. Likewise, serum ADD was suppressed by 50%, but serum DHEA was unchanged.

Discussion: ADD and DHEA are highly concentrated within the human testes compared to serum. Serum and IT-ADD and IT-DHEA are markedly suppressed with GnRH administration and stimulated by hCG, but serum DHEA is not, suggesting that most circulating DHEA is not of testicular origin.

Sources of Research Support: The National Institute of Child Health and Human Development supported this work through cooperative agreements U54 HD-12629 and U54 HD-42454 as part of the Specialized Cooperative Centers Program in Reproductive Research and the Cooperative Contraceptive Research Centers Program. Dr. Roth is supported by the Male Reproductive Health Research Award, K12 HD053984. Dr. Page is supported by the National Institute of Aging, a Division of the National Institute of Health, by grant K23 AG027238. Dr. Matsumoto is supported by the Department of Veterans Affairs.

Disclosures: AMM: Principal Investigator, GlaxoSmithKline; Investigator, Solvay Pharmaceuticals, Inc.; Editor, Up To Date. Nothing to Disclose: MYR, STP, KL, BDA, BM, WJB, JKA

Pub # P1-329

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Changes in Reproductive Hormone Concentrations Predict the Prevalence and Progression of the Frailty Syndrome in Older Men: The Concord Health and Aging in Men Project

Author String TG Trivison, A-H Nguyen, DJ Handelsman
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Body **Background:** Frailty, a syndrome of multiple morbidities, weakness, and immobility in aging, is an urgent threat to public health (1). Single measures of low serum androgen have demonstrated suggestive associations with the frailty syndrome (2-4), but the contributory role of **within-subject hormonal changes with time** has not been assessed.

Objective: To determine, using longitudinal measurements, the contributions of serum androgens, estrogens, gonadotropins, and sex hormone-binding globulin (SHBG) to the prevalence and progression of frailty in older men.

Methods: The CHAMP study cohort is a representative sample of 1645 men (age 70+) living in Concord, Sydney, Australia (5). All measures were conducted at baseline (2005-07) and two-year followup (2007-09). The major androgens testosterone (T) and dihydrotestosterone (DHT), and estrogens estradiol (E2) and estrone (E1), were measured by liquid chromatography-tandem mass spectrometry. SHBG and the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone were measured by immunoassay. Frailty was assessed using both the Cardiovascular Health Study (CHS) index by Fried and colleagues (6) and Study of Osteoporotic Fractures (SOF) index by Ensrud and colleagues (7); each categorizes subjects as either 'robust', 'pre-frail' or 'frail.' Analyses employed logistic and proportional odds regression via generalized estimating equations (8).

Results: The frailty indices exhibited moderate subject-level agreement (intraclass correlation coefficient ~ 0.5); independently, each indicated that 41% of subjects were pre-frail and 9% frail. Androgens and estrogens showed uniformly significant age-adjusted associations with frailty status measured by either index. Subjects in the lowest T quintile had 2.2-fold odds of exhibiting more significant CHS frailty as compared to the highest T quintile ($p < 0.001$); results for DHT, E2, E1 and calculated free T were similar, and were unchanged when SOF frailty was substituted for the CHS index. A one standard deviation two-year decrease in T, FT or LH was associated with a 1.2 to 1.3-fold increase in the odds of concurrent progression (increase in severity) of frailty, whereas baseline estrogen levels were (inversely) associated with frailty development or progression. Controlling for the presence of comorbid medical conditions did not affect results.

Conclusions: Age-related decreases in blood androgens and estrogens may accelerate the development of frailty in men.

- (1) Xue CL, Clin Geriatr Med 2011; 27:1
- (2) Mohr B et al., J Am Geriatr Soc 2007; 55:548
- (3) Cawthon PM et al., J Clin Endocrinol Metab 2009; 94:3806
- (4) Hyde Z et al., J Clin Endocrinol Metab 2010; 95:3165
- (5) Cumming RG et al., Int J Epidemiol 2009; 38:374
- (6) Fried LP et al., J Gerontol A Biol Sci Med Sci 2001; 56:M146
- (7) Ensrud KE et al., Arch Intern Med 2008; 168:382
- (8) Zeger, SL and Liang KY, Biometrics 1986; 42:121

Sources of Research Support: NHMRC Grant 301916, University of Sydney Medical Foundation.

Nothing to Disclose: TGT, A-HN, DJH

Pub # P1-330

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Androgen Receptor CAG Repeat Polymorphism Modulates Change in Triglycerides, Diastolic Blood Pressure and PSA during Testosterone Replacement Therapy in Men with Metabolic Syndrome or Type 2 Diabetes - The TIMES2 Study

Author String RD Stanworth, S Akhtar, KS Channer, JD Howell, TH Jones
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Body Background. Testosterone replacement therapy (TRT) has beneficial effects on insulin resistance and other cardiovascular risk markers in men with type 2 diabetes and other patient groups.^{1,2} The androgen receptor CAG repeat (ARCAG) polymorphism alters the transcriptional response of the androgen receptor to ligand such that shorter AR CAG is associated with greater activity.
Objective. To evaluate the effect of ARCAG on response to TRT in hypogonadal men with type 2 diabetes and/or metabolic syndrome.
Research design and Methods. A multicentre, European prospective, randomised, double-blind, placebo-controlled study of transdermal 2% testosterone gel over 12 months was conducted in 220 hypogonadal men with metabolic syndrome and/or type 2 diabetes (TIMES2 study). Primary efficacy analysis focused on 6 month time point during which time no changes to medications were permitted. The results of this trial have been presented elsewhere and demonstrated beneficial effects on insulin resistance (HOMA-IR), total and LDL cholesterol and lipoprotein-a.² All participants consented to assessment of ARCAG at the start of the trial but consent was deemed to be withdrawn in those who withdrew. ARCAG was assessed by sequence analysis in 147 men of whom 76 were from the TRT group. Multivariate linear regression models were constructed for change in variable y after 6 months TRT. Predictor variables were ARCAG, baseline testosterone, change in testosterone, baseline waist, change in waist, age and baseline variable y.
Results. ARCAG was significantly related to change in fasting triglycerides ($r=0.221$, $p=0.011$), diastolic blood pressure ($r=0.258$, $p=0.025$) and PSA ($r=-0.253$, $p=0.037$). There was a trend to a relationship between ARCAG and change in fasting insulin ($r=0.201$, $p=0.065$). There was no significant relationship of ARCAG with change in HOMA-IR, fasting glucose, HbA1C, lipid fractions (other than triglycerides), systolic blood pressure, obesity or haematocrit. Baseline testosterone was significantly related to change in haematocrit ($r=-0.301$, $p=0.012$) but not other variables and change in testosterone was not significantly related to any variables.
Conclusion. Shorter, more active AR CAG are associated with favourable changes in triglycerides, diastolic blood pressure and fasting insulin as well as greater rises in PSA during 6 months TRT in men with type 2 diabetes and/or metabolic syndrome. Lower baseline testosterone predicted greater increase in haematocrit on TRT.

1. Kapoor D et al., Eur J Endocrinol 2006;154:899-906

2. Jones TH et al., Diabetes Care 2011;In press

Sources of Research Support: ProStrakan; On behalf of the TIMES2 investigators.

Disclosures: JDH: Employee, Pro Strakan. Nothing to Disclose: RDS, SA, KSC, THJ

Pub # P1-331

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Men with Low Testosterone and Type 2 Diabetes Have Reduced Health-Related Quality of Life (HRQoL)

Author String DJ Walter, JC Brooke, V Muraleedharan, D Kapoor, M Hazel, TH Jones
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Body Hypogonadism is prevalent in men with type 2 diabetes.[1] We are unaware of any reports on HRQoL in hypogonadal type 2 diabetics.
We have previously published that in a cohort of 356 men with type 2 diabetes, 17% had overt hypogonadism and a further 25% were hypogonadal with low normal testosterone levels.[1] As part of the screening process in this study, the SF36 HRQoL questionnaire was completed. [1, 2] Total (TT) and bioavailable (BT) testosterone were measured and free testosterone (cFT) was calculated using Vermeulens' equation. HRQoL data was analysed using PASW software. Where SF36 questions were not answered, the total HRQoL score was not calculated, but if all questions for an individual domain were present this was included in the analysis. The mean baseline characteristics of the cohort (n=356) were age 57.9 (± 10.1) years and TT 12.7 (± 5.5) nmol/l. Mean BT and cFT were 4.03 (± 1.4) nmol/l and 0.26 (± 0.12) nmol/l respectively. Of the 356 questionnaires, 315 were valid for total HRQoL scoring. Scores were adjusted to have a potential range of 0-100. The mean total SF36 score was 69.1 (± 16.4) with a cohort range of 27.3 to 97.9.
Raw TT data correlated positively with the total SF36 score (0.123, $p < 0.05$) as well as with physical (0.135, $p < 0.05$), social function (0.141 $p < 0.01$), vitality (0.118, $p < 0.05$) and health change over time (0.145, $p < 0.01$) but lost significance when adjusted for covariates. BT was found to correlate with total HRQoL ($r = 0.137$, $p < 0.05$) and domains of general ($r = 0.109$, $p < 0.05$), vitality ($r = 0.124$, $p < 0.05$), social ($r = 0.105$, $p < 0.05$) and physical health ($r = 0.177$, $p < 0.01$) but not independent of covariates.
Linear regression analysis revealed that cFT correlates with total HRQoL ($r = 0.411$, $p = 0.029$) when adjusted for BMI, HbA1c and cardiovascular co-morbidities. Under the same analysis, cFT also correlated with physical ($r = 0.440$, $p = 0.023$), social ($r = 0.335$, $p = 0.039$) and vitality ($r = 0.304$, $p = 0.012$) domains and health change over time ($r = 0.220$, $p = 0.003$).
In men with type 2 diabetes, cFT levels have a significant impact on HRQoL in the total scores of the SF36 questionnaire. They also have an impact on physical, social and vitality domains and health change over time scores.

1. Kapoor D et al., Diabetes Care 2007; 30:911-7
2. Ware J.E. Jr et al., Journal of Clinical Epidemiology 1998; 51:903-12

Sources of Research Support: Barnsley Hospital Endocrinology Research Fund.

Nothing to Disclose: DJW, JCB, VM, DK, MH, THJ

Pub # P1-332

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Severity of Erectile Dysfunction and Testosterone Deficiency Are Associated with Reduced Quality of Life (HRQoL) in Men with Type 2 Diabetes Mellitus

Author String JC Brooke, DJ Walter, V Muraleedharan, D Kapoor, M Hazel, TH Jones
University of Sheffield, Sheffield, UK; Barnsley Hospital NHS Foundation Trust, Barnsley, UK

Body There is a high prevalence of hypogonadism and erectile dysfunction (ED) amongst men with Type 2 diabetes (1). Low testosterone levels have an impact on the severity of ED(2). Evidence to show associations between testosterone levels, severity of ED and HRQoL in diabetic men is currently lacking. Testosterone deficiency and ED are both independently correlated with increased risk of cardiovascular disease so any findings will be of important clinical significance.

We investigated the effect of low testosterone and ED on HRQoL in a cohort of 356 men with Type 2 diabetes. Total testosterone (TT), bioavailable testosterone (BT) and sex hormone binding globulin (SHBG) were measured from morning blood samples and free testosterone (cFT) levels were calculated using Vermeulen's equation. 356 patients completed SF-36 and ADAM questionnaires and a subgroup of 126 patients with ED also completed short form IIEF questionnaires.

Mean demographics: age 58 years (± 10.1), TT 12.7nmol/L (± 5.55), BT 4.0nmol/L (± 1.49), cFT 274pmol/L (± 125), IIEF score 11.8 (± 4.64). SF-36 scores ranged from 27.9 to 100, with a mean of 70.6 (± 16.8). Regression analyses were corrected for age, BMI, HbA_{1c}, smoking, alcohol consumption and cardiovascular disease using PASW software.

A t-test revealed that patients who reported having ED on the ADAM questionnaire had an average SF-36 (HRQoL) score of 9.1 points less than those without ED (95% CI 5.2 to 13, $p < 0.001$). Total testosterone levels significantly correlated with total SF-36 scores when corrected for SHBG ($r = 0.35$, $p = 0.044$).

Within the subgroup of patients who completed IIEF questionnaires, IIEF scores significantly correlated with total SF-36 scores ($r = 0.49$, $p = 0.003$). IIEF scores also correlated with SF-36 domains: Physical ($r = 0.50$, $p = 0.003$), Physical role limitations ($r = 0.35$, $p = 0.031$), Social ($r = 0.45$, $p = 0.022$), Vitality ($r = 0.38$, $p = 0.025$), Pain ($r = 0.43$, $p = 0.012$), and General health ($r = 0.41$, $p = 0.001$). IIEF scores significantly correlated with levels of TT (corrected for SHBG) ($r = 0.55$, $p < 0.001$), BT ($r = 0.51$, $p = 0.004$) and cFT ($r = 0.53$, $p < 0.001$).

This is the first study to report that lower testosterone and severity of ED are both independently associated with reduced HRQoL in diabetic men. The results also show that severity of ED is related to testosterone levels in diabetic men. Since both of these factors are known to be associated with cardiovascular disease, these results highlight the need for clinicians to be aware of ED and hypogonadism.

(1) Kapoor D et al., Diabetes care 2007; 30(4):911-7.

(2) Kapoor D et al., Int J Androl 2007; 30(6):500-7.

Sources of Research Support: Barnsley Hospital Endocrinology Research Fund.

Nothing to Disclose: JCB, DJW, VM, DK, MH, THJ

Pub # P1-333

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Low Testosterone Predicts Development of Diabetes among Men at High but Not Low Metabolic Risk

Author String AB Araujo, V Kupelian, GA Wittert
New England Research Institutes, Inc, Watertown, MA; The University of Adelaide, Adelaide, Australia

Body Type 2 diabetes mellitus (T2DM), an increasing health and economic burden worldwide, is a major cause of premature illness and death. In population studies of normally aging men, low testosterone (T) levels have been linked with T2DM. We tested whether T is associated with incident T2DM among men at varying risk of developing T2DM, defined by risk factors for metabolic syndrome (MetS) and body mass index (BMI). Data were obtained from the Massachusetts Male Aging Study (MMAS), a population-based, prospective study of 1,709 men (aged 40-70 years at baseline) observed at 3 timepoints over a period of approximately 15 years (T1 (baseline): 1987-89; T2: 1995-97; T3: 2002-04). T was assessed by radioimmunoassay. T2DM was assessed by self-reported physician diagnosis. Available risk factors for MetS included: high blood pressure (self-report or measured $>130/85$ mmHg or BP medication use), low high-density lipoprotein cholesterol (<40 ng/mL), elevated waist circumference (>102 cm). Metabolic risk was classified using number of components of MetS (0-1, 2, or 3) and BMI (<25 , 25-29, ≥ 30). Poisson regression of incident T2DM on continuous total T overall and within metabolic risk strata was performed to estimate relative risks (RR) and 95% confidence intervals (95% CI). There were N=1130 men with no T2DM at baseline, who were observed at T2 or T3 with data on T2DM status. Mean baseline age and T was 53.8 y (SD=8.3) and 523 ng/dL (SD=173), respectively. Results showed an increase in risk of incident T2DM with decreasing levels of Total T. However, this association varied across metabolic risk strata with an association of larger magnitude observed in men with higher risk of T2DM (multiple components of MetS and/or obesity) while no association was observed in men with 0-1 components of MetS or non-obese men. For instance, there was an increasing trend in the RR (per 100 ng/dL decrease in T) for incident T2DM from 0.98 (95% CI: 0.80, 1.20) among men with 0-1 MetS components, 1.26 (95% CI: 0.96, 1.64) among men with 2 MetS components, and 1.41 (95% CI: 1.04, 1.91) among men with 3 MetS components. The risk of T2DM associated with total T increases monotonically as MetS risk score increases, suggesting that there is an interaction between T levels and metabolic risk in predicting T2DM and also supporting the potential for T to have a role in prevention of conversion of MetS to T2DM, which should be tested in intervention trials.

Nothing to Disclose: ABA, VK, GAW

Pub # P1-334

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Obese Men with Both Elevated Estradiol and Low Testosterone Levels Have an Adverse Cardiovascular Risk Profile

Author String T Ahern, M Corrigan, E O'Malley, C Dunlevy, D O'Shea, F Hayes
St Vincent's University Hospital, Dublin, Ireland; St Vincent's University Hospital, Dublin, Ireland; St Columcille's Hospital, Loughlinstown, Ireland

Body

Introduction
Obese men have lower testosterone (T) and higher oestradiol (E₂) levels than similarly aged men of normal weight, due to a number of factors including increased conversion of T to E₂ by adipose tissue aromatase. Low T levels are associated with an increased incidence of cardiovascular disease (CVD) in men. In contrast, cross-sectional studies in men have linked high E₂ levels with CVD. We therefore hypothesized that obese men with *both* low T *and* elevated E₂ levels have the greatest CVD risk.

Methods
The following cardiovascular risk factors were assessed in 129 obese men (mean BMI 51.4 kg/m², mean age 47 yr): serum C-reactive-protein (hsCRP) concentration, homeostatic model of insulin resistance (HOMA-IR) value, glycosylated haemoglobin (HbA1c) and fitness test times. The fitness tests consisted of ascending and descending a step 50 times and walking 1 km. We calculated the 10-year risk of developing CVD using the Framingham Heart study multivariate risk factor algorithm. Research participants were stratified into four groups based on their sex hormone levels: normal T, normal E₂ (n= 32); low T, normal E₂ (n=33); low T, high E₂ (n=30); and normal T, high E₂ (n=34). Data are expressed as medians. Kruskal-Wallis analyses were used to determine whether differences existed between groups.

Results
The median Framingham Risk score for the entire cohort was 13.4% and did not differ between groups. Compared to obese men with normal sex hormone levels, those with *both* low T *and* elevated E₂ concentrations had the worst levels of:
- inflammation (hsCRP 11.4 vs 3.1 mg/L, p<0.0001);
- glycaemia (HbA1c 6.1 vs 5.8%, p=0.03); and
- fitness (1km walk time 15.9 vs 12.2 min, p=0.05
50 step test time 2.5 vs 1.9 min, p=0.03).
Insulin resistance was significantly greater (p=0.006) in men with *either* a low T (HOMA-IR 8.5) *or* an elevated E₂ concentration (HOMA-IR 8.4) than in those with normal sex hormone levels (HOMA-IR 5.4).

Conclusions
In this cohort of severely obese middle-aged men, CVD risk is high and only 25% have normal levels of both T and E₂. Perturbations in the sex hormone milieu are associated with increased inflammation, glycaemia and insulin resistance and with lower physical fitness. Based on these data, we propose that interventions targeted to normalise *both* low T *and* elevated E₂ levels are likely to ameliorate these CVD risk factors.

Sources of Research Support: The Irish Heart Foundation in the form of an unrestricted research grant.

Nothing to Disclose: TA, MC, EO, CD, DO, FH

Pub # P1-335

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Aromatase Inhibition in Healthy Men Induces Insulin Resistance, Elevated Blood Pressure, and Altered Plasma Lipids, with Limited Changes in Transcript Levels in Subcutaneous Adipose Tissue

Author String FW Gibb, KJ McInnes, R Andrew, BR Walker
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Body Congenital aromatase deficiency in humans and mice is associated with central obesity and insulin resistance, putatively mediated by intra-adipose estrogen deficiency and/or androgen excess. We tested whether pharmacological, acquired inhibition of aromatase has adverse metabolic effects, and investigated potential mechanisms in adipose tissue, the major site of extra-gonadal aromatase expression. Seventeen healthy men (age 28 ± 3 y; BMI 25.7 ± 1.1 kg/m²) received placebo or oral anastrozole (1 mg daily for 6 weeks) in a double-blind randomized crossover study with a 2 week 'washout'. At the end of each phase, blood was taken after overnight fast and a euglycemic hyperinsulinemic clamp was performed. In subjects with adequate subcutaneous abdominal adipose depots, tissue was obtained by needle biopsy; apart from higher BMI and age, these subjects (n=6) were representative of the larger group. mRNAs were quantified using real-time PCR, with cyclophilin as internal control. Data are presented as mean \pm SEM and compared by paired Student's t-tests. Anastrozole did not affect body fat, but increased systolic blood pressure (133.6 ± 2.9 vs 138.4 ± 2.5 mmHg, $p < 0.05$), reduced total cholesterol (4.12 ± 0.13 vs 3.86 ± 0.13 mM, $p < 0.05$) and tended to lower HDL cholesterol (by 0.08 mM, $p = 0.07$). Anastrozole lowered insulin sensitivity during low dose (10mU/m²/min), but not high dose (40mU/m²/min), insulin infusion (steady state glucose infusion rate to maintain euglycemia 2.55 ± 0.24 vs 2.19 ± 0.27 mg/kg/min, $p = 0.02$). In subcutaneous adipose tissue, anastrozole reduced transcript levels of estrogen receptor beta (1.8-fold, $p < 0.04$) and perilipin (10%, $p < 0.05$) and tended to increase mRNA for genes involved in cholesterol metabolism (SREBP2 and downstream HmGCoA synthase), but had no effect on mRNA for adipokines (leptin, adiponectin), fatty acid metabolism (LPL, HSL, CETP, ACCa1, DGAT2, FASN), adipose regulation (PPAR γ or PGC-1 α , α - or β -adrenoceptors, SREBPF1, β -catenin) or steroid signalling (ER α , AR, aromatase). We conclude that acquired loss of function of aromatase in healthy men has adverse cardiometabolic effects and induces changes in gene transcription in adipose tissue that are consistent with intra-adipose estrogen deficiency. However, the lack of more striking alterations in subcutaneous adipose gene expression suggests that the metabolic effects are mediated by inhibition of aromatase either in the gonads or in other adipose depots.

Sources of Research Support: Wellcome Trust.

Disclosures: BRW: Investigator, Wyeth Pharmaceuticals; Ad Hoc Consultant, Boehringer Ingelheim; Astra Zeneca. Nothing to Disclose: FWG, KJM, RA

Pub # P1-336

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Population-Based Reference Ranges for Testosterone in Men Derived Using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) and Validated in Three Geographically Distinct Cohorts

Author String S Bhasin, MM Pencina, GK Jasuja, TG Travison, AD Coviello, EM Orwoll, PY Wang, CM Nielson, FM Wu, AM Tajar, HM Vesper, AM Zhang, JM Ullor, RM Singh, RM D'Agostino, RS Vasani
Boston University School of Medicine, Boston, MA; Boston University School of Public Health, Boston, MA; Oregon Health Sciences University, Portland, OR; University of Manchester, Manchester, MA; Centers for Disease Control, Atlanta, GA; Mayo Clinic, Rochester, MN; Boston University School of Medicine, Boston, MA

Body Androgen deficiency in men is characterized by a constellation of symptoms and signs and low circulating testosterone (T) levels. Rigorously established reference ranges are essential for identifying whether T levels are normal or low. Population-based reference ranges for T using reliable assays are not available. Objective: To generate reference limits for total (TT) and free testosterone (FT) concentrations in a community-based sample of healthy young men in the Framingham Heart Study (FHS) Generation 3 cohort. Methods: TT was measured using liquid chromatography tandem mass spectrometry (LC-MS/MS), SHBG by an immunofluorometric assay, and FT was calculated. TT and FT values below the 2.5th percentile were deemed low. For validation, we applied these reference limits to three geographically distinct cohorts of community-dwelling men: FHS generations 2 and 3, the European Male Aging Study (EMAS), and the Osteoporotic Fractures in Men Study (MrOS). We determined whether men in these 3 cohorts with low TT or FT by the proposed reference limits had a higher prevalence of 3 conditions that have been consistently associated with low T levels: physical dysfunction, sexual symptoms, and diabetes. We used thresholds based on a healthy young reference sample (T-score approach) because in exploratory analyses, the T-score approach and age-adjusted thresholds (Z-score approach) yielded concordant results for most outcomes. Spline plots of T levels against outcomes in the FHS sample did not yield clear inflection points. Results: In the reference sample of 456 men, mean(SD), median(quartile), and 2.5th percentile values were 724(221), 699(297), and 348ng/dL for TT and 142(45), 134(60), and 70pg/mL for FT, respectively. In validation samples, men with low TT and FT were more likely to have slow walking speed, difficulty climbing stairs, or frailty, and diabetes than those with normal levels. In EMAS, men with low TT and FT were more likely to report sexual symptoms than men with normal levels. Conclusion: Population-based reference ranges provide a rational basis for categorizing T levels as [ldquo]low [rdquo] or [ldquo]normal[rdquo]. Men with low TT or FT levels by these criteria had higher prevalence of physical dysfunction, sexual dysfunction, and diabetes. These reference limits should be validated prospectively in relation to incident outcomes and in prospective randomized trials.

Sources of Research Support: NIH grant 1R01AG31206; Boston Claude D. Pepper Older Americans Independence Center grant 5P30AG031679; a grant from the CDC Foundation.

Nothing to Disclose: SB, MMP, GKJ, TGT, ADC, EMO, PYW, CMN, FMW, AMT, HMV, AMZ, JMU, RMS, RMD, RSV

Pub # P1-337

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Prevalence of Hypogonadism in Men with Diabetes Using Laboratory-Specific Reference Range for Serum Testosterone

Author String M Biswas, D Hampton, A Rees
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Body

Background:
Hypogonadism has a reported prevalence rate of up to 30% in men with type 2 diabetes. Previous studies have used various biochemical cut offs to define hypogonadism and may have included patients in whom testosterone levels were affected by other illnesses or drugs.
Using our own laboratory-assay specific reference range for serum total testosterone, we assessed the prevalence of hypogonadism in diabetic men aged 20-70 years attending our hospital clinic.

Methods:
Reference ranges for serum total testosterone in men aged 20-70 y were determined for the Abbot Architect testosterone immunoassay from 120 healthy male white Caucasian volunteers recruited from hospital staff and medical students. All subjects had fasting glucose < 5.9 mmol/l, waist circumference < 94 cm, alcohol intake < 3 units per night, no significant illness and denied recreational drug or medication use.
Men aged 20 -70 years attending our hospital diabetes clinic filled in the IIEF questionnaire and had blood drawn between 0830 and 1030am for total testosterone measurement.
Patients were excluded if they were currently affected with chronic obstructive airways disease, infection, depression, renal or liver disease, cancer, inflammatory joint or bowel disease, pituitary, testicular, adrenal disease, abnormal thyroid function or if they had been admitted to hospital within the previous month. Any patient who had previously received or was currently taking anabolic steroids, testosterone, corticosteroids, ketoconazole or spironolactone was also excluded.
A score of less than 25/30 on the IIEF questionnaire indicated the presence of erectile dysfunction. Total testosterone of less than 8nmol/l was defined as hypogonadal.

Results:
A total of 208 diabetic men were studied, (n=115 type 2, n=93 type 1). Mean age was 51.4 y \pm 11.76 and mean duration of diabetes was 14.96 y \pm 11.37.
Based on the revised laboratory cut-off, 21 were identified as biochemically hypogonadal, 19/115 (16.5%) patients with type 2 diabetes and 3/93 (3.2%) patients with type 1 diabetes. 71% of all patients studied reported erectile dysfunction.

Conclusion:
Using laboratory specific biochemical criteria and excluding patients with illnesses and or taking drugs which may affect serum testosterone levels, we found that diabetes related hypogonadism was less prevalent than previously quoted. Erectile dysfunction was common and largely unrelated to low testosterone levels.

Nothing to Disclose: MB, DH, AR

Pub # P1-338

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title The Relationship between Sex Hormones, Sex Hormone Binding Globulin and Peripheral Artery Disease in Older Persons

Author String C Cattabiani, G Ceda, F Lauretani, S Bandinelli, G Schiavi, A Vignali, R Volpi, G Ceresini, A Artoni, L Ferrucci, M Maggio
University of Parma, Parma, Italy; University Hospital, Parma, Italy; Azienda Sanitaria Firenze, Florence, Italy; National Institutes of Health (NIH), Baltimore, MD

Body Peripheral artery disease (PAD) is an important and multi-factorial condition in older individuals. The prevalence of PAD increases with age and concurs to the development of frailty and disability. Fewer investigations have assessed the role of sex hormones and sex hormone binding globulin (SHBG) in PAD (1-2).
Aim of the Study. To test the association between sex hormones, SHBG and lower extremity PAD in the elderly.
Methods. 933 older men and women with complete data on SHBG, total testosterone (T) were selected from the InCHIANTI study. SHBG and T were measured using immunoradiometric assay with minimum detectable concentration (MDC) of 3.0 nmol/lit and 0.08 nmol/lit, respectively. The inter- and intraassay CV concentrations were < 3.7, and 11.5 for SHBG and < 9.6 and 9.1, for T. Total Estradiol (E2) was measured using ultrasensitive radioimmunoassay with a MDC of 2.2 pg/mL and intra- and interassay CVs <10%. The Ankle-Brachial Index (ABI) was measured with a hand-held Doppler stethoscope. PAD was defined as an ABI <0.90 and absence of PAD by ABI > 0.90. Logistic regression models adjusted for age (Model 1), for age, BMI, interleukin-6, physical activity, smoking, hypertension, diabetes, chronic heart failure, HDL-cholesterol (Model 2) and Model 3 including also T, SHBG and total E2 were used to test the association between SHBG sex hormones (predictors) and PAD (outcome).
Results. The mean age \pm SD of the 933 (419 men and 514 women) subjects at baseline was 71.8 ± 4.9 years (74.2 ± 6.5 in men and 75.6 ± 7.0 in women). 64 (43 men and 21 women) participants had ABI <0.90 and 865 participants ABI > 0.90. Only in men, SHBG was positively associated with PAD in both Model 1 (OR: 3.24, CI 1.46-7.18, $p=0.0038$) and Model 2 (OR 3.58, CI 1.36-9.40, $p=0.009$), while this relationship did not reach the statistical significance in Model 3 ($p=0.06$).
Total E2 was not significantly associated with PAD in both men ($p=0.68$) and women ($p=0.21$). In women, T was positively associated with PAD, in Model 1 (OR 5.93, CI 1.29-27.11, $p=0.02$), Model 2 (OR 1.72, CI 0.99-2.97, $p=0.053$), and this relationship was still significant in Model 3 (OR 2.048, CI 1.13- 3.72, $p=0.01$). In contrast, in men, T was not independently associated with PAD.
Conclusion
Higher SHBG and T are significantly associated with PAD in older men and women, respectively. Further longitudinal studies are needed to address the role of SHBG and T in the development of PAD in the elderly.
1. Price JF, Lee AJ, Fowkes FG. Steroid sex hormones and peripheral arterial disease in the Edinburgh Artery Study. *Steroids* 1997;62: 789-94.
2. Tivesten A, Mellstr[ouml]m D, Jutberger H, Fagerberg B, Lernfelt B, Orwoll E, Karlsson MK, Ljunggren O, Ohlsson C. Low serum testosterone and high serum estradiol associate with lower extremity peripheral arterial disease in elderly men. The MrOS Study in Sweden. *J Am Coll Cardiol*. 2007 Sep 11;50(11):1070-6.
Sources of Research Support: The InCHIANTI Study was supported as a "targeted project" (ICS 110.1/RS97.71) by the Italian Ministry of Health and in part by the U.S. National Institute on Aging (Contracts N01-AG-916413 and N01-AG-821336) and by the Intramural Research Program of the U.S. National Institute on Aging (Contracts 263 MD 9164 13 and 263 MD 821336).
Nothing to Disclose: CC, GC, FL, SB, GS, AV, RV, GC, AA, LF, MM

Pub # P1-339

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Sex Hormone Binding Globulin Is a Marker of Hepatic Insulin Sensitivity and Lipogenesis, but Is Not Independently Associated with Type 2 Diabetes Mellitus in Men

Author String G Wittert, S Martin, D Wilson, A Jenkins, A Araujo, P O'Loughlin, A Taylor, R Adams
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Body Sex hormone binding globulin (SHBG), synthesized in hepatocytes, is inversely associated with the metabolic syndrome, and reported to predict type 2 diabetes mellitus (T2DM). In vitro the SHBG promoter is regulated by HNF-4 (which also regulates pancreatic beta cell function and which is defective in MODY-4 is itself regulated by thyroid hormone and pathways involved in de-novo lipogenesis). We examined the relationships between SHBG and body composition (DEXA), metabolic state, thyroid hormones, sex steroids hsCRP and IL6 at baseline and after 5 years as well as incident T2DM (based on fasting glucose, HbA1c, doctor diagnosis or medication use) in a prospectively-followed, randomly selected cohort of community dwelling men aged 35-80 at enrolment (n=1195). After excluding pre-existing diabetes (n=112), thyroid disease (n=25), prostate cancer (n=27) and use of exogenous glucocorticoids (n=47), sex steroids (n=14) or other medications known to affect SHBG (n=12), data from 937 men were available at baseline and 705 at 5 years. T, DHT and E2 from both time points were measured concurrently by LC-MS/MS; SHBG, insulin, T3 and T4 by solid-phase, two-site chemiluminescent immunoassay and CRP and IL6 by ELISA. Data were adjusted for age and smoking and further evaluated by multivariate analysis. At baseline there were independent positive associations between SHBG and age($\beta=.342$, $p=.000$), T, $(\beta=.439$, $p=.000)$ DHT($\beta=.224$, $p=.000$), T4 ($\beta=.067$, $p=.013$)and TSH ($\beta=.086$, $p=.001$) and inverse associations between SHBG and triglycerides ($\beta=-.127$, $p=.000$) and abdominal fat mass ($\beta=-.052$, $p=.045$), with no relationship to smoking, insulin, glucose, E2, CRP or IL6. There were 74 incident cases of T2DM. Baseline SHBG did not predict the onset of T2DM independent of change in BMI, waist circumference, smoking, or change in level of ALT, IL6, CRP, or sex steroids. Incident diabetes was predicted by higher age (OR: 1.03, 95%CI: 1.00-1.06), abdominal fat mass (1.03; 1.00-1.07), fasting plasma glucose (2.24; 1.56 - 3.24) and estradiol (1.030; 1.009-1.051) and lower testosterone (0.80; 0.62 - 0.93). These data are consistent with the notion that low SHBG reflects an unfavourable metabolic state reflective of insulin resistance and de novo lipogenesis. There is no direct effect of insulin on SHBG, and SHBG is not in and of itself associated with T2DM risk.

Sources of Research Support: NH&MRC project grant 627227.

Nothing to Disclose: GW, SM, DW, AJ, AA, PO, AT, RA

Pub # P1-340
Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)
Title Elevated Luteinizing Hormone Predicts Ischemic Heart Disease Events in Older Men
Author String Z Hyde, PE Norman, L Flicker, GJ Hankey, KA McCaul, OP Almeida, P Chubb, BB Yeap
University of Western Australia, Crawley, Australia; University of Western Australia, Crawley, Australia;
University of Western Australia, Crawley, Australia; Royal Perth Hospital, Perth, Australia; University of
Western Australia, Crawley, Australia; Royal Perth Hospital, Perth, Australia; Fremantle Hospital, Fremantle,
Australia; Fremantle Hospital, Fremantle, Australia

Body

Context

Hypogonadism in men is associated with insulin resistance, elevations in pro-inflammatory cytokines and fibrinogen, and an atherogenic lipid profile. However, it is uncertain whether the age-related decline in testosterone is associated with ischaemic heart disease (IHD) events.

Objective

To determine whether testosterone, and its associated hormones, sex hormone binding globulin (SHBG) and luteinizing hormone (LH), predict IHD events.

Design

Prospective cohort study.

Methods

Between 2001-04, 3,637 community-dwelling men aged 70-88 years underwent a clinical assessment of cardiovascular risk factors, and biochemical assessment of testosterone, SHBG, and LH. Free testosterone was estimated. Participants were followed until December 2008 using electronic record linkage to capture IHD events (hospital admission or death).

Results

Mean follow-up was 5.1 years. During this period, 618 men (17.0%; 95% CI 15.8-18.3%) experienced an event, of which 160 were fatal. Men with higher baseline total or free testosterone levels experienced fewer IHD events (HR=0.89; 95% CI 0.82-0.97 and HR=0.86; 95% CI 0.79-0.94 for each one standard deviation increase in total and free testosterone, respectively). These associations were maintained after adjustment for age and waist:hip ratio, but did not persist after adjustment for prevalent IHD or other cardiovascular risk factors. SHBG was not associated with IHD events. In contrast, higher LH levels were associated with greater IHD events in both univariate (HR=1.15; 95% CI 1.08-1.22) and adjusted analyses (HR=1.08; 95% CI 1.01-1.15).

Conclusions

Dysregulation of the hypothalamic-pituitary-gonadal axis may be a risk factor for IHD. Further studies of men with either elevated LH or low testosterone are warranted.

Sources of Research Support: National Health and Medical Research Council of Australia (grant numbers 279408, 379600, 403963, 513823 and 634492); MBF Foundation of Australia (grant number DS 080608); NHMRC Biomedical Postgraduate Scholarship to ZH; Sylvia and Charles Viertel Charitable Foundation Clinical Investigator Award to BY; Fremantle Hospital Medical Research Foundation grant to PC.

Nothing to Disclose: ZH, PEN, LF, GJH, KAM, OPA, PC, BBY

Pub # P1-341

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Prevalence and Predictors of Sexual Dysfunction in Older Men

Author String Z Hyde, L Flicker, GJ Hankey, KA McCaul, OP Almeida, P Chubb, BB Yeap
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Body

Context
Hypogonadism in younger men is associated with impaired libido and erectile dysfunction. Testosterone levels decline with age, but the relationship between androgens and sexual dysfunction in older men is controversial.

Objective
To determine whether testosterone levels are associated with sexual dysfunction.

Design
Prospective cohort study.

Setting
Perth, Western Australia.

Participants
1,744 community-dwelling men aged 70-88 years (mean 76 years) at baseline.

Methods
Questionnaires in 2001-04 and 2008-09 assessed social and medical factors. Testosterone, SHBG and LH were measured in 2001-04. Sexual dysfunction was assessed by questionnaire in 2008-09 (mean follow-up period 5.2 years).

Results
Sexual problems were highly prevalent, with 50.5% (95% CI 48.2-52.9%) reporting erectile dysfunction, 46.5% (95% CI 44.2-48.8%) lacking interest in sexual activity, 38.4% (95% CI 36.1-40.6%) unable to climax and 22.0% (95% CI 20.1%-24.0%) anxious about their ability to perform sexually. Painful and unpleasurable sex were less common (<5%). In multivariate logistic regression analyses, total testosterone levels in the lowest quintile were associated with lack of interest (OR=1.59; 95% CI 1.13-2.23), but were not associated with any other sexual problem. Cardiovascular disease, diabetes, and insomnia were the factors most commonly associated with sexual problems.

Conclusions
Androgen deficiency is unlikely to be a major cause of sexual dysfunction in older men. However, low testosterone levels may be a causal factor in impaired libido. Clinical trials should investigate this concept.

Sources of Research Support: National Health and Medical Research Council of Australia (grant numbers 279408, 379600, 403963, 513823 and 634492); MBF Foundation of Australia (grant number DS 080608); NHMRC Biomedical Postgraduate Scholarship to ZH; Sylvia and Charles Viertel Charitable Foundation Clinical Investigator Award to BY; Fremantle Hospital Medical Research Foundation grant to PC.

Nothing to Disclose: ZH, LF, GJH, KAM, OPA, PC, BBY

Pub # P1-342

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Clinical Relevance of Identifying Late-Onset Hypogonadism Based on Proposed Criteria

Author String YKD Tay, KK Ng, R Chen
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Body

Aim
It has been proposed that late-onset hypogonadism (LOH) may be identified in older males based upon total testosterone (TT) <11 mM and free testosterone (FT) <220 pM in the presence of poor morning erections, reduced libido and erectile dysfunction. As testosterone deficiency is reportedly associated with metabolic syndrome (MetS), we sought to study the relevance of this proposed criteria in clinical practice.

Method
Cross-sectional study of 236 consecutive males, predominantly Chinese (mean age 54.6±7.6 (SD) years), self-referred to a hospital-based Men's Health clinic for perceived symptoms of androgen deficiency. Sex hormones, fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG) and high-density lipoprotein (HDL) were assayed; low-density lipoprotein (LDL) and FT calculated; blood pressure (BP), waist circumference (WC) and body mass index (BMI) obtained. Subjects also answered the modified International Index of Erectile Dysfunction (IIEF-5) and Ageing Male Symptom (AMS) questionnaires. MetS was defined using the ethnic specific International Diabetes Federation guidelines.

Results
The prevalence of LOH and MetS was 8.4% and 36.5%, respectively. Men with LOH had significantly poorer IIEF-5 scores (10.1±4.5 vs 13.9±5.9, P=0.006), higher WC (94.9±8.3 vs 89.1±9.1 cm, P=0.011), BMI (27.6±3.4 vs 24.9±3.2 kg/m², P=0.000) and FPG (7.4±2.8 vs 6.0±1.4 mM, P= 0.048); lower TT (8.6±1.2 vs 16.2±4.9 nM, P= 0.000), FT (173.7±24.4 vs 314.6±74.9 pM, P=0.000) and sex hormone binding globulin (30.4±6.8 vs 37.7±16.7 nM, P=0.000). However, the prevalence of MetS was not significantly different between groups with and without LOH (46.7% vs 35.7% P=0.396). Neither was BP (systolic 134.4±14.8 vs 129.2±18.5 mmHg, P=0.236; diastolic 81.0±10.9 vs 78.5±10.8 mmHg, P=0.353), TC (5.3±1.3 vs 5.4±0.9 mM, P=0.790), TG (2.1±2.5 vs 1.6±0.9 mM, P=0.437), HDL (1.3± 0.3 vs 1.4±0.4 mM, P=0.526), LDL (3.1±1.0 vs 3.2±0.9 mM, P=0.430) nor total AMS scores significantly different (43.9±10.4 vs 41.1±11.4, P=0.306).

Conclusion
While the proposed criteria identify hypogonadal men with sexual dysfunction, it does not appear to identify those with MetS or parameters of poor metabolic status apart from WC, BMI and FPG, suggesting that it may be of limited clinical relevance.

(1) Wu FC et al. Identification of late-onset hypogonadism in middle-aged and elderly men. *N Engl J Med* 2010;363(2):123-35.

Nothing to Disclose: YKDT, RC

Pub # P1-343

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Estradiol Levels Predict Bone Mineral Density in Male Collegiate Athletes

Author String KE Ackerman, GS Skrinar, E Medvedova, M Misra, KK Miller
Massachusetts General Hospital, Boston, MA; Boston University, Boston, MA

Body

Purpose: Strenuous training can result in amenorrhea and bone loss in female collegiate athletes. However, the impact of athletic training on endocrine function and bone mineral density (BMD) in male collegiate athletes is less well understood. The objective of this study was to investigate the specific determinants of BMD in male collegiate athletes, including the impact of gonadal steroid levels.

Methods: In this cross-sectional study of 26 division I collegiate male athletes, wrestlers, runners, and golfers (who served as controls), participants performed grip and maximal oxygen intake (VO₂ max) testing, provided blood samples, and had full body dual energy x-ray absorptiometry (DXA) scans. Main outcome measures included 1) BMD endpoints measured by DXA; 2) endocrine endpoints: total and free estradiol, total and free testosterone; 3) body composition endpoints: fat-free and fat mass, measured by DXA; and 4) exercise endpoints: VO₂ max, number of miles run weekly, and grip strength.

Results: Mean BMD was significantly higher in the wrestlers than the runners and controls at all skeletal sites. Free and total estradiol levels were significant positive determinants of BMD (free estradiol: R=0.49, 0.57, and 0.61 at the PA and lateral spine and radius, respectively; total estradiol R=0.41, 0.48, and 0.58). In contrast, total and free testosterone levels were not significant predictors of BMD at any skeletal site (with the exception of free testosterone at the radius). In addition, lean mass, % ideal body weight, total body weight, BMI, and hours per week of resistance training were significant positive predictors of BMD (R=0.40-0.74). VO₂ max was a significant negative predictor of BMD (R=-0.61 and -0.55 at the PA and lateral spine, respectively.) Using stepwise multivariate modeling, fat-free mass accounted for 37%, VO₂ max 28%, and free estradiol 5% of the variability of PA spine BMD. Fat-free mass accounted for 44%, VO₂ max 22%, and free estradiol 6% of the variability of lateral spine BMD. Fat-free mass accounted for 44% and free estradiol 19% of the variability of radius BMD. Fat-free mass accounted for 24% of the variability of total hip BMD and 26% of the variability of femoral neck BMD.

Conclusions: Our data suggest that estradiol levels, lean mass, and VO₂ max are important determinants of BMD in male collegiate athletes, but that testosterone does not appear to be an important factor.

Sources of Research Support: NIH Grant MO1 RR01066.

Nothing to Disclose: KEA, GSS, EM, MM, KKM

Pub # P1-344

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title The Relative Bioavailability of Testosterone after Administration of 1.62% Testosterone Gel Using Different Methods of Application

Author String J Stahlman, MG Miller, C McWhirter, SA Testino, Jr, TL Zumbrennen
Abbott, Marietta, GA

Body
Objective: The objective of this study was to determine the application regimen pharmacokinetics and relative bioavailability of testosterone after application of a 1.62% testosterone gel (T-gel) in hypogonadal males using different application methods.
Methods: Sixty-two hypogonadal men (serum total testosterone <300 ng/dL) participated in this randomized, open-label, 2-way crossover study with a 7-day washout period. Application methods consisted of application of 5.0 g of 1.62% T-gel application once daily to the abdomen for the first 3 days followed by the upper arms/shoulders for the last 4 days of the 7-day treatment period (Method A) and once daily to a combination of both the abdomen (2.5 g) and upper arms/shoulders (2.5 g) for 7 days (Method B). Serial blood samples were collected at baseline (day -1 and 14), and after 7 consecutive days of gel application with each method for pharmacokinetic assessments. Testosterone concentrations in each sample were determined using validated LC/MS/MS methodology.
Results: Both application methods resulted in mean time-averaged testosterone concentrations (C_{av}) within the eugonadal range (300-1000 ng/dL). The mean [SD] maximum serum concentration (C_{max}) was within the eugonadal range for method B (866 [369] ng/dL) and was slightly above the eugonadal range (>1000 ng/dL) for method A (1283 [817] ng/dL) using a fixed dose of 5.0 g. Mean testosterone C_{av} and C_{max} were lower in magnitude for method B, however the bioavailability of method B was comparable to that of method A. The 1.62% T-gel was safe and well tolerated for both application methods.
Conclusion: Both gel application methods provided clinically relevant testosterone concentrations in hypogonadal males. These study results support dosing with 1.62% T-gel by either alternating between the abdomen and upper arms/shoulders application sites or by applying to a combination of both the abdomen and upper arms/shoulders.

Sources of Research Support: Abbott.

Disclosures: JS: Employee, Abbott Laboratories. MGM: Employee, Abbott Laboratories. CM: Employee, Abbott Laboratories. SAT: Employee, Abbott Laboratories. TLZ: Employee, Abbott Laboratories.

Pub # P1-345

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Low Estradiol Concentrations in Males with Hypogonadotropic Hypogonadism and Type 2 Diabetes

Author String S Dhindsa, R Furlanetto, M Vora, H Ghanim, P Dandona
State University of New York at Buffalo, Buffalo, NY; Quest Diagnostics, Chantilly, VA

Body One-third of men with type 2 diabetes have hypogonadotropic hypogonadism(HH). It has been suggested that HH in these men may be due to an increase in plasma estradiol(E2) concentrations secondary to an increase in aromatase activity in the adipose tissue which leads to the suppression of hypothalamo-hypophyseal-gonadal(HHG) axis. We investigated the hypothesis that plasma E2 concentrations are significantly greater in type 2 diabetic males with HH as compared to those without HH. Plasma estradiol, testosterone(T), LH and sex hormone binding globulin(SHBG) concentrations were measured in fasting blood samples of 236 men with type 2 diabetes (mean age: 56±12; range:23-83 years; mean BMI: 35±7;range:17-59kg/m²) attending a tertiary diabetes referral center. Total T was measured by liquid chromatography tandem mass spectrometry(LC-MS/MS). In 196 men, total E2 was measured by an immunoassay. Free E2 and T concentrations were calculated using total E2, T, albumin and SHBG. In 99 men, total E2 was measured by the more specific LC-MS/MS assay. Free E2 and free T concentrations in these men were measured by tracer equilibrium dialysis. HH was defined as free T<3.5 ng/dl along with LH<10 IU/L. E2 concentrations between men with and without HH were compared after adjusting for age and BMI differences. The calculated free E2 concentration in men with HH was lower than that in eugonadal men (median 0.047[0.035-0.068] vs. 0.063 [0.046-0.077] ng/dl, p<0.001). Directly measured free E2 concentrations were also lower in men with HH (median 0.023[0.008-0.049] vs 0.045[0.027-0.069] ng/dl, p=0.01). In multiple regression analysis, free E2 was independently and directly related to free T(β =0.24, p=0.001) but not to BMI(β =0.10, p=0.17) or age (β =-0.07, p=0.37). Since total and free E2 concentrations are significantly lower in HH males, the syndrome of HH cannot be due to an excess of E2. The diminished E2 concentration appears to be a function of its precursor, T. The pathogenesis of HH in type 2 diabetes needs to be investigated further.

Sources of Research Support: NIH Grants R01-DK075877 and R01-DK069805 awarded to PD; American diabetes Grant awarded to PD; American Diabetes Grant 10-JF-13 awarded to SD.

Disclosures: SD: Speaker, Abbott Laboratories. PD: Principal Investigator, GlaxoSmithKline; Clinical Researcher, Sanofi-Aventis; Speaker, Novartis Pharmaceuticals; Eli Lilly & Company; GlaxoSmithKline; Sanofi-Aventis. Nothing to Disclose: RF, MV, HG

Pub # P1-346

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Pharmacokinetics of Modified Slow-Release Oral Testosterone over 10 Days in Normal Men with Experimental Hypogonadism

Author String AP Lee, K Rubinow, RV Clark, RB Caricofe, MA Bush, H Zhi, MY Roth, ST Page, WJ Bremner, JK Amory
University of Washington, Seattle, WA; GlaxoSmithKline, Research Triangle Park, NC

Body

Background: Oral administration of testosterone might be useful for the treatment of male testosterone deficiency but acceptable formulations are currently unavailable in the United States. We have recently demonstrated that a novel formulation of oral testosterone designed to slow absorption from the gut improved hormone pharmacodynamics in a single-dose pharmacokinetic study (1). We hypothesize that thrice daily dosing of this novel drug would normalize free testosterone in experimentally hypogonadal men.

Methods: To further characterize the steady-state pharmacokinetics and pharmacodynamics of this novel formulation, twelve healthy young men were rendered hypogonadal by the administration of the gonadotropin-releasing hormone (GnRH) antagonist acyline (300 mcg/kg SQ) and administered 300 mg of modified slow-release oral testosterone three times daily for 9 days. Measurements of serum testosterone, DHT, estradiol, and sex-hormone binding globulin (SHBG) were obtained before and 1, 2, 4, 6, 8, 10, 12, 14, 16 and 24 hours after the morning dose on the first and ninth day of drug administration.

Results: Prior to the first dose of testosterone, all 12 men had castrate testosterone levels with average levels less than 50 ng/dl. The geometric mean (%CV) serum total testosterone was 378 (45%) ng/dl over the first 24 hour sampling period, but declined to 302 (39%) ng/dl after 10 days of continued treatment (p= 0.1). The geometric mean serum SHBG was 27 (46%) nmol/l over the first 24-hour sampling period and was significantly reduced during the second 24-hour sampling period to 20 (47%) (P <0.01), resulting in similar values for the calculated free testosterone between the two study periods. Serum DHT was slightly elevated and serum estradiol remained in the normal range. Adverse events were minimal. All subjects except one showed a peak testosterone level less than two times the upper limit of normal.

Conclusions: We conclude that 300 mg of oral modified slow-release testosterone dosed three times daily increases serum testosterone to the low-normal range in men with experimentally-induced hypogonadism. Total testosterone levels declined with continued dosing in the steady state, but free testosterone did not, likely secondary to suppression of serum SHBG concentrations. This formulation of oral testosterone might have efficacy for the treatment of male testosterone deficiency.

1. Snyder C et al. Pharmacokinetics of 2 novel formulations of modified-release oral testosterone alone and with finasteride in normal men with experimental hypogonadism. *J Androl.* 2010 Nov-Dec;31(6):527-35

Sources of Research Support: The Eunice Kennedy Shriver National Institute of Child Health and Human Development, a division of the National Institute of Health through cooperative agreement U54 HD42454 as part of the Cooperative Contraceptive Research Centers Program and GlaxoSmithKline. A portion of this work was conducted through the Clinical Research Center facility at the University of Washington and supported by the NIH grant UL1-RR-025014.

Disclosures: RVC: Employee, GlaxoSmithKline. RBC: Employee, GlaxoSmithKline. MAB: Employee, GlaxoSmithKline. HZ: Employee, GlaxoSmithKline. Nothing to Disclose: APL, KR, MYR, STP, WJB, JKA

Pub # P1-347

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Testosterone Replacement Therapy in Male Hypogonadism: Final Results from the Largest International Substitution Trial Involving 1493 Patients

Author String M Zitzmann, JU Hanisch, A Mattern, M Maggi
University Clinics, Muenster, Germany; Bayer Schering Pharma Inc, Berlin, Germany; University of Florence, Florence, Italy

Body

Introduction & objectives: Safety and efficacy are pivotal in testosterone replacement therapy of hypogonadal men. Benefits in terms of improved mental, sexual and metabolic health have to be achieved by this regimen. Especially in older patients, the balance between such benefits and potential adverse aspects has to be carefully elucidated.

Material & methods: Final results from the largest world-wide multi-center non-interventional, surveillance study assessing the safety and effectiveness of injectable long-acting testosterone undecanoate (TU) in hypogonadal men in daily clinical practice receiving 6333 injections during 1103 patient years are presented. TU therapy was administered for typically 9-12 months. Patient data from 23 countries in Europe, Asia, Latin America and Australia were acquired. Prostate safety was closely monitored by digital rectal examination and assessment of PSA levels. Prostate biopsies were taken according to standard of care at discretion of the investigator. Parameters of erectile function, libido, vigour/vitality, mood and ability to concentrate were assessed by physician interview using items and five-point Likert scales from validated tools. Physical and circulatory parameters as well as hematocrit, glucose control and lipid profiles were recorded.

Results: Overall, scores of mental and sexual satisfaction (libido, vigour, overall mood, ability to concentrate increased markedly (all $p < 0.0001$), while mean waist circumference decreased significantly from 100 cm to 96 cm ($p < 0.0001$). These results were stable upon stratification between men receiving previous androgen therapy and those who were not previously treated. The percentage of patients who reported [ldquo]low [rdquo] or [ldquo]very low[rdquo] levels of sexual desire/libido decreased from 64% at baseline to 10%. Subjects with elevated HbA1c levels (cut-off $> 6.1\%$) exhibited a decline from 7.9% to 6.8% ($p < 0.0001$). PSA levels and hematocrit remained within the normal range, no case of prostate cancer was observed. Mean PSA levels increased from 1.1 ± 0.9 ng/ml to 1.3 ± 1.2 ng/ml and remained stable ($p < 0.0001$). PSA exceeded 4 ng/ml in 11 men. No case of prostate cancer was observed.

Conclusion: These results from the largest worldwide sample of hypogonadal men receiving substitution therapy by injectable TU document that this form of therapy is well tolerated in daily clinical practice, extending previous findings especially in regard to psychological and metabolic efficacy as well as safety.

Nothing to Disclose: MZ, JUH, AM, MM

Pub # P1-348

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Intramuscular Testosterone Undecanoate for Substitution in Male Hypogonadism -- The Experience of 13.5 Years Elucidates Beneficial Effects on the Newly Defined Metabolic Syndrome and Reveals a High Degree of Safety

Author String M Zitzmann, F Saad
University Clinics, Muenster, Germany; Bayer Schering Pharma Inc, Berlin, Germany

Body

Background:
A reliable form of testosterone substitution with favourable kinetics and tolerance is important for hypogonadal men. Intramuscular injections of long-acting testosterone undecanoate (TU) offer a convenient modality.

Methods:
We report data from 281 patients (134 with primary, 88 with secondary hypogonadism and 59 with late-onset ([ldquo]mixed[rdquo] or [ldquo]metabolic[rdquo] hypogonadism) aged 15 to 72 years (mean 40±13 years) receiving altogether 4913 intramuscular injections of 1000 mg of TU during a maximal treatment time of 13.5 years, overall corresponding to 1069 treatment years.
Components of the metabolic syndrome were assessed in 216 men receiving 2864 injections.

Results:
Trough levels of testosterone were generally within the low normal range, indicating sufficient substitution. Individual dosing intervals ranged from 10 to 14 weeks. The proportion of men fulfilling the new joint consensus criteria of the International Diabetes Federation and the National Cholesterol Education Program for definition of the Metabolic Syndrome decreased from initially 87% to 52% within 2 years (Chi-square for trend:p<0.001). Especially waist circumference decreased from 112.0±10.3 to 96.4±9.1 cm (p<0.001) within 1 year and body mass index decreased from 30.4±5.1 to 28.6±3.8 kg/m² (p=0.001). Lipoprotein subfractions, blood pressure and fasting glucose levels improved in a similarly significant manner. Hematocrit was significantly elevated under treatment but remained within the normal range, except for occasional measurements (maximal value 56.3%).

Conclusion:
Injections of testosterone undecanoate represent a feasible, safe and well tolerated modality of androgen substitution in hypogonadal men of a wide age-range, substantiated by more than one decade of experience, facilitating a decrement of metabolic/cardiovascular risk factors.

Nothing to Disclose: MZ, FS

Pub # P1-349

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title The Long-Term Effect of Testosterone Replacement Therapy on Adipocytokines and Metabolic Parameters in Male Hypogonadism

Author String JH An, JH Kim, JA Lim, KW Kim, SW Kim, CS Shin, SY Kim
Seoul National University College of Medicine, Seoul, Republic of Korea; Seoul National University Hospital, Seoul, Republic of Korea; Seoul National University Boramae Hospital, Seoul, Republic of Korea

Body
Background: Low levels of testosterone in men have been known to be associated with metabolic syndrome. We investigated the long-term effect of testosterone replacement therapy on metabolic parameters and various adipocytokines in hypogonadal men.
Methods: We enrolled 71 men who were diagnosed with primary or secondary hypogonadism and started replacement of oral testosterone (testosterone undecanoate) from January 2000 to December 2007. Metabolic parameters, plasma adiponectin, leptin, retinol-binding protein-4 (RBP-4) and resistin were measured every year.
Results: A total of 71 hypogonadal men (mean age, 43.0 ± 14.8 years) were treated with oral testosterone for 6.2 ± 2.1 years. At baseline, total testosterone levels were significantly correlated with adiponectin ($r=0.37$), leptin ($r=-0.48$), RBP-4 ($r=-0.33$), and resistin ($r=-0.45$) levels (all $p<0.05$). Plasma leptin levels progressively decreased (baseline 19.1 ± 4.3; 1-year 15.1 ± 4.2; 5-year 11.5 ± 3.9 ng/ml, $p<0.05$), and resistin levels progressively increased (baseline 16.0 ± 6.2; 1-year 20.2 ± 6.8; 5-year 28.4 ± 8.2 ng/ml, $p<0.05$) after testosterone replacement therapy. We did not observe any significant change in plasma adiponectin and RBP-4 levels during 5 years of treatment. Serum total cholesterol, LDL-cholesterol, and HDL-cholesterol levels were significantly decreased after 1 year of treatment, and there were no further significant change up to 5 years. Body mass index, waist circumference, blood pressure, fasting glucose and triglyceride levels were not changed.
Conclusion: Long-term testosterone replacement therapy in hypogonadal men had favorable effects on leptin and LDL-cholesterol levels. However, resistin and HDL-cholesterol levels were negatively affected by testosterone replacement.

Nothing to Disclose: JHA, JHK, JAL, KWK, SWK, CSS, SYK

Pub # P1-350

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Serum Testosterone Levels in Non-Dosed Females after Secondary Exposure to 1.62% Testosterone Gel: Effects of Clothing Barrier on Testosterone Absorption

Author String J Stahlman, M Britto, S Fitzpatrick, C McWhirter, SA Testino, Jr, JJ Brennan, TL Zumbrunnen
Abbott, Marietta, GA; Quintiles, Inc, Overland Park, KS

Body
Objective: To determine the pharmacokinetics of testosterone transferred to females from a male partner, dosed with 1.62% testosterone gel (T-gel) after direct skin-to-skin contact with the application site, and to investigate the effect of wearing a t-shirt on testosterone transfer.
Methods: Across 3 studies, a total of 72 healthy males applied 5.0 g 1.62% T-gel to their abdomen alone, upper arms/shoulders alone, or to both the upper arms/shoulders and abdomen (single dose or daily for 7 days). Male-female contact occurred 2 or 12 hours after T-gel application, with males either wearing or not wearing a t-shirt. Blood samples were collected in females for 24-hour assessments of serum testosterone levels at baseline and after contact using validated LC/MS/MS methodology. Pharmacokinetic values assessed included maximum serum concentration (C_{max}) and time-averaged concentration over the 24-hour period post contact (C_{av}). Subjects were monitored for adverse events.
Results: Testosterone levels (C_{av} and C_{max}) in females increased 86-185% from baseline after direct abdominal skin contact, although C_{av} levels remained within the female eugonadal range (8-75 ng/dL). Testosterone concentrations returned to baseline within 48 hours after the last skin contact. A t-shirt barrier reduced testosterone transfer by approximately 40-48% when 5.0 g of T-gel was applied to the 2 sites on the abdomen alone. A t-shirt barrier prevented transfer when 5.0 g of T-gel was applied to the upper arms and shoulders (2 sites) alone or to the upper arms and shoulders and both sides of the abdomen (4 sites in total). No major safety events were observed during the studies.
Conclusions: There is a risk of testosterone transfer from males using 1.62% T-gel to others who come into contact with the application site for at least 12 hours after application. Secondary exposure can be mitigated by means of a t-shirt barrier.

Sources of Research Support: Abbott.

Disclosures: JS: Employee, Abbott Laboratories. MB: Researcher, Abbott Laboratories. SF: Employee, Abbott Laboratories. CM: Employee, Abbott Laboratories. SAT: Employee, Abbott Laboratories. JJB: Employee, Abbott Laboratories. TLZ: Employee, Abbott Laboratories.

Pub # P1-351

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Effect of Application Site, Clothing Barrier and Application Site Washing on Testosterone Transfer with a 1.62% Testosterone Gel

Author String J Stahlman, M Britto, S Fitzpatrick, C McWhirter, SL Testino, Jr, JJ Brennan, TL Zumbrunnen
Abbott, Marietta, GA; Quintiles, Inc, Overland Park, KS

Body
Objectives: To evaluate the effect of application site, clothing barrier and application site washing on the transfer of testosterone from males dosed with 1.62% testosterone gel (T-gel) to female partners.
Methods: An open-label, randomized, parallel group, crossover study was performed in 24 healthy male/female couples. Up to 5.0 g of gel was applied to the upper arms and shoulders or abdomens of male subjects. Skin contact occurred 2 hours after gel application between male and female subjects to compare the effect of wearing or not wearing a t-shirt and washing or not washing before contact. Serum samples were collected from females for 24-hour assessments of testosterone levels using validated LC/MS/MS methodology; maximum serum concentration (C_{max}), and time-averaged concentration over the 24-hour post contact period (C_{av}) were assessed. Subjects were monitored for adverse events.
Results: Testosterone exposure (C_{av} and C_{max}) in females increased by up to 27% (2.5 g) or up to 280% (5.0 g) from baseline after direct skin contact at 2 hours with the gel application site on the male partner, although C_{av} remained within the eugonadal range. Transfer after direct skin contact was higher when the application and contact sites were the upper arms/shoulders versus the abdomen. Transfer from the abdomen was prevented when a t-shirt was worn (2.5 g dose). When the application site was washed before contact, mean C_{av} was comparable to baseline and C_{max} was slightly higher (14%). Testosterone concentrations returned to baseline within 48 hours after last skin contact. The 1.62% T-gel appeared to be safe and well tolerated in this study.
Conclusions: There is a risk of testosterone transfer from males using 1.62% T-gel to others who come into direct skin contact with the application site. This can be prevented by covering the application site with a t-shirt or washing the site of gel application before anticipated contact.

Sources of Research Support: Abbott.

Disclosures: JS: Employee, Abbott Laboratories. MB: Researcher, Abbott Laboratories. SF: Employee, Abbott Laboratories. CM: Employee, Abbott Laboratories. SLT: Employee, Abbott Laboratories. JJB: Employee, Abbott Laboratories. TLZ: Employee, Abbott Laboratories.

Pub # P1-352

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Pharmacokinetics, Efficacy, and Safety of Testosterone Topical Solution 2% Applied to the Axillae in Androgen-Deficient Men

Author String C Wang, N Ilani, S Arver, RI McLachlan, T Soulis, AC Watkinson
Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Torrance, CA; Karolinska Universitetssjukhuset Huddinge, Stockholm, Sweden; Prince Henry's Institute of Medical Research, Clayton, Australia; Acrux, West Melbourne, Australia

Body Testosterone (T) replacement therapy is administered to hypogonadal men to relieve symptoms and normalize serum T levels to within the reference range for healthy men. Testosterone topical solutions are effective in the treatment of androgen deficiency. We studied the safety, pharmacokinetics (PK), and efficacy of a novel testosterone topical solution 2% applied daily to skin in both axillae.

This 120-day open-label trial was conducted at 26 centers in 6 countries in androgen-deficient men (serum T <300 ng/dL). At Days 15, 60, and 120, testosterone PK measurements over 24 h were taken by liquid chromatography tandem mass spectrometry and an average T level calculated. Subjects started on a daily dose of 60 mg (1.5 mL applied to each axilla). Dose adjustments to maintain serum T levels within the physiologic range (300-1050 ng/dL), if necessary, were done at Days 45 and 90 based on average serum T levels on Days 15 and 60, respectively. Sexual function and mood changes were assessed by the Psychosexual Daily Questionnaire (PDQ) and quality of life by SF-36 questionnaire. Safety parameters, laboratory tests, and adverse events (AEs) were collected at each visit.

In total, 155 subjects were enrolled (mean age 51.5 years). Of 135 study completers (and 3 who withdrew due to AEs), 76.1% (Day 15/16), 84.8% (Day 60/61), and 84.1% (Day 120/121) had an average total T level between 300-1050 ng/dL. Among subjects in eugonadal range at day 120 (responders), the geometric mean serum T value for subjects on any dose was 486 ng/dL. PDQ mean sexual desire and overall sexual activity scores increased by 79% and 103%, respectively (both p<0.0001). PDQ mean summary scores showed significant increases in positive mood and decreases in negative mood (both p<0.0001). Subjects had significant improvement in the physical (p<0.05) and mental components (p<0.0001) of the SF-36. AEs reported in >5% of subjects were application site irritation (7.7%), application site erythema (5.2%), and headache (5.2%). Three serious AEs were reported, none of which were considered by the investigators to be treatment-related (appendicitis, hepatitis C, and prostate cancer).

Testosterone topical solution 2% applied to the axillae normalized serum T in 84% of androgen-deficient men assessed and significantly increased psychosexual health. Once-daily application of testosterone topical solution 2% in both axillae was a well-tolerated and efficacious treatment for androgen replacement in hypogonadal men.

Sources of Research Support: Research grants from Acrux Pty. Ltd. (Melbourne, Australia).

Disclosures: CW: Principal Investigator, Acrux Australia; Investigator, GlaxoSmithKline; Repros, Clarus; Consultant, Lilly USA, LLC. SA: Speaker, Eli Lilly & Company; Pfizer, Inc.; Bayer, Inc.; Astra Zeneca; Consultant, Acrux Australia; Bayer, Inc.; Ferring Pharmaceuticals; Pfizer, Inc.; Sanofi-Aventis; Research Funding, Bayer, Inc. RIM: Speaker, Schering Plough; Consultant, Acrux Australia; Bayer Australia Pty. Ltd.; Research Funding, Bayer Schering Pharma. TS: Employee, Acrux Australia. ACW: Employee, Acrux Australia. Nothing to Disclose: NI

Pub # P1-353

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Baseline Body Mass Index (BMI) Has No Effect upon Normalization of Testosterone Concentrations with Testosterone 2% Gel

Author String A Dobs, J McGettigan, P Norwood, S Potts, E Gould
The Johns Hopkins University, Baltimore, MD; Quality of Life Medical and Research Center, Tucson, AZ; Valley Endocrine and Valley Research, Fresno, CA; Endo Pharmaceuticals, Chadds Ford, PA

Body Objective: Obesity can influence levels of serum total testosterone (STT) especially in aging males. Little is known how weight affects testosterone (T) replacement therapy (TRT). This post hoc analysis of an efficacy data set from a phase 3 trial shows effectiveness and safety of T2% gel in hypogonadal males (HM) and effects of BMI on TRT. Methods: During a 90d, phase 3, open-label, non-comparative trial, 129 HM patients with STT concentration <250ng/dL or 2 consecutive STT concentrations <300ng/dL received T2% gel (Fortesta[®]) as TRT. BMI measurements were collected at baseline. Study medication was applied once daily to thighs at starting doses of 40mg/day using a unique metered dose delivery system adjustable in 10mg increments. On Days 14, 35 and 60 doses were kept constant or adjusted to between 10 and 70mg/day according to predefined criteria depending on STT concentrations obtained 2h after application. Endpoints were average STT concentrations over 24h (C_{avg} 0-24h) and maximum STT concentration (C_{max}) at 90d. Study objective was to raise TC_{avg} 0-24h to a normal range of [ge]300 and [le]1140ng/dL in [ge]75% patients Results: At baseline, 8 patients (6%) had normal weight (BMI [ge]18.5-[le]24.9kg/m²; Group A); 43 patients (33%) were overweight (BMI [ge]25-[le]29.9kg/m²; Group B); and 78 patients (61%) were obese (BMI [ge]30kg/m²; Group C). Mean STT levels at baseline were 199.8±92.1ng/dL, 190.3±69.4ng/dL and 198.5±63.1ng/dL (A/B/C, respectively). At 35d, TC_{avg} 0-24h was 409.1±152.7ng/dL, 414.9±133.3ng/dL and 404.6±166.4ng/dL (A/B/C, respectively). At 90d, TC_{avg} 0-24h was 457.86±154.0ng/dL, 433.4±171.5ng/dL and 439.5±160.2ng/dL (A/B/C, respectively). TC_{max} at 90d was 947.4±269.6ng/dL, 821.8±341.1ng/dL and 818.6±373.6ng/dL (A/B/C, respectively). Patients (%) with STT concentration in the normal range at 35d were 75.0, 87.8 and 70.1% (A/B/C, respectively) and at 90d, 75.0, 76.7 and 78.2% (A/B/C, respectively). At 90d these were 100.0, 95.3 and 93.6% (A/B/C, respectively), with C_{max} values [le]1500ng/dL. Mean average daily T2% gel dose was 45.4±5.4mg, 42.7±7.3mg and 46.3±7.4mg (A/B/C, respectively). The T2% gel was generally well tolerated and most common adverse incidents were application site reactions considered mild (79%) to moderate (21%). Conclusions: In HM using T2% gel, at 90d, TC_{avg} 0-24h and C_{max} were similar in patients with normal, overweight and obese BMI. Baseline BMI did not predict daily doses of T2% gel required to maintain STT levels within the expected range.

Sources of Research Support: Endo Pharmaceuticals.

Disclosures: SP: Employee, Endo Pharmaceuticals. EG: Employee, Endo Pharmaceuticals. Nothing to Disclose: AD, JM, PN

Pub # P1-354

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology I (1:30 PM-3:30 PM)

Title Normal Physiologic Testosterone Concentrations Were Obtained with Testosterone 2% Gel in Previously Treated and Treatment-Naive Hypogonadal Males

Author String A Dobs, J McGettigan, P Norwood, S Potts, E Gould
The Johns Hopkins University, Baltimore, MD; Quality of Life Medical and Research Center, Tucson, AZ; Valley Endocrine and Valley Research, Fresno, CA; Endo Pharmaceuticals, Chadds Ford, PA

Body Objective: Testosterone (T) replacement therapy (TRT) in hypogonadal males (HM) previously treated for low T levels may be less effective compared with TRT in treatment-naive (TN) patients. This post hoc analysis of an efficacy data set in a phase 3 study looked at differences in treatment effect between previously treated and TN patients. Methods: In a phase 3, open-label, non-comparative trial, 129 HM patients--with single serum total T (STT) concentrations <250ng/dL or 2 consecutive STT concentrations <300ng/dL--received T2% gel (Fortesta[trade]) for 90d. Patients with previous TRT had a washout period of length dependent on prior TRT, before baseline STT measurements were taken. Study medication was applied once daily to thighs at starting doses of 40mg/day using a unique metered dose delivery system adjustable in 10mg increments. On Days 14, 35 and 60 doses could be altered to between 10 and 70mg/day according to predefined criteria depending on STT concentrations obtained 2h after application. Endpoints were average STT concentrations over 24h (C_{avg} 0-24h) and maximum STT concentration (C_{max}) at 90d. Study objective was to raise TC_{avg} 0-24h to a normal range of [ge]300 and [le]1140ng/dL in [ge]75% patients. Results: At baseline, 89 patients (69%) had prior TRT (Group A) and 40 patients (31%) were TN (Group B). Mean STT levels at baseline were 192.1±67.7ng/dL (Group A) and 204.1±64.7ng/dL (Group B). At 35d, TC_{avg} 0-24h was 393.8±152.7ng/dL (Group A) and 439.2±155.5ng/dL (Group B). At 90d, TC_{avg} 0-24h was 435.4±162.9ng/dL (Group A) and 445.5±163.4ng/dL (Group B). TC_{max} at 35d was 800.0±387.4ng/dL (Group A) and 872.4±401.9ng/dL (Group B) and at 90d was 862.9±349.6ng/dL (Group A) and 829.3±376.1ng/dL (Group B). Patients (%) with normal range STT values at 35d were 69.8 and 90.0% (A/B, respectively); at 90d 77.5% of patients in both groups had normal range STT values. At 35d, 93.0 and 95.0% (A/B, respectively) had C_{max} values [le]1500ng/dL and at 90d 94.4 and 95.0% (A/B, respectively). The T2% gel was generally well tolerated with most common adverse incidents being application site reactions considered mild (79%) to moderate (21%). Conclusions: At 90d TC_{avg} 0-24h and C_{max} values were similar in TN patients and those who had prior TRT. At 90d 77.5% of patients in both groups had STT values in the normal range and nearly all had TC_{max} levels [le]1500ng/dL. No treatment differences were seen between previously-treated and TN HM after 90d TRT with T2% gel.

Sources of Research Support: Endo Pharmaceuticals.

Disclosures: SP: Employee, Endo Pharmaceuticals. EG: Employee, Endo Pharmaceuticals. Nothing to Disclose: AD, JM, PN

Pub # P1-355

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Potential Role of Ventral Premammillary Nucleus in Female Sexual Behavior

Author String J Donato, Jr, CR Franci, NS Canteras, CF Elias
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Body The ventral premammillary nucleus (PMV) neurons express androgen receptor and estrogen receptor alpha. PMV neurons are responsive to mating and to conspecific odorants that are relevant for the induction of motivated behaviors. PMV densely projects to sexually dimorphic nuclei that regulate the reproductive axis and sexual behaviors. Although the role of PMV neurons in regulating the neuroendocrine reproductive axis has been investigated, no study assessed a potential role of PMV in modulating sexual behaviors. In order to determine a potential role of PMV in female sexual behavior, we induced bilateral excitotoxic lesions of the PMV in cycling female rats. Ten weeks later, we assessed their sexual behavior in the afternoon of proestrus day (3 hours after lights off). Females were housed with sexually experienced males, and their behavior was recorded following the first 10 attempts of mount. They were classified as high responsive (9-10 lordosis), intermediate (2-6 lordosis) or non-responsive (0 lordosis). Thirty min after the beginning of the test, blood samples were collected and rats were perfused. The lesions were determined by thionin staining and by expression of NADPH diaphorase activity and CART mRNA. We obtained 5 rats with bilateral lesions of the PMV (PMV-lesioned group) and 11 control animals. We observed that PMV-lesioned rats showed no change in the expression of proceptive behaviors. However, compared to the control group, we found that a high percentage of PMV-lesioned rats were non-responsive (40% vs.18%) and only a small percentage of PMV-lesioned rats were high responsive (20% vs.46%). We also observed that 30% of males which mated with control rats reached ejaculation, whereas none of them ejaculated when mating with PMV-lesioned rats. No significant changes were observed in the concentrations of LH, FSH, prolactin or estradiol. But we found a positive correlation between progesterone levels and the expression of lordosis. In situ hybridization analysis indicated that, at the level of the OVLT, the number of GnRH neurons and GnRH mRNA expression/cell were increased in PMV-lesioned rats. On the other hand, lesions of PMV suppressed Kiss1 mRNA expression in the AVPV and the PeN. Our results suggest that the PMV is required for full expression of lordosis behavior in cycling female rats. We propose that this effect is secondary to changes in the fine control of the neuroendocrine components that regulate the reproductive system.

Sources of Research Support: NIH grant HD061539 (to C.F.E.), and by São Paulo Research Foundation (FAPESP-Brazil, 05/58997-4 and 05/59286-4).

Nothing to Disclose: JD, CRF, NSC, CFE

Pub # P1-356

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Analysis of Estrogen Signaling Pathways in Zebrafish Eye during Optic Nerve Regeneration

Author String AT McCurley, RK Meyer, PN Francois, GV Callard
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Body In addition to a role in neurodevelopment and neuroplasticity, estrogen synthesized in the nervous system itself is reported to have neuroprotective effects and could also be a factor in neurodegenerative disorders and age-related neural decline. Due to the diversity and complexity of the mammalian nervous system, a comprehensive mechanistic understanding of neuroestrogen's role in neuronal survival and repair remains a challenge. In contrast to mammals, retinal ganglion cells (RGC) of teleost fish, including the zebrafish (*Danio rerio*), display a robust regenerative response after optic nerve crush (ONX), such that vision is restored in ~21 d. Furthermore, as compared to other vertebrates, fish retina and other neural tissues express extraordinarily high levels of aromatase enzyme and mRNA, suggesting exaggerated functions. To begin to address this hypothesis, microarray (Affymetrix) and quantitative (Q)PCR analysis were used for a detailed time course analysis of gene expression patterns in eye after ONX (1). A specific functional link between neuroestrogen and neuroregeneration was then investigated in ONX fish treated without/with ICI 182,780 (estrogen receptor antagonist) or fadrozole (aromatase inhibitor). ONX upregulated aromatase B (*cyp19a1b*, estrogen biosynthesis marker) mRNA ~2.5-fold at 6 hr but not other time points. Aromatase A (*cyp19a1a*) and estrogen receptor (ER)- α , - β a, and - β b (*esr1*, *2a*, *2b*) mRNAs were not significantly changed by ONX. ICI and fadrozole decreased *cyp19a1b* mRNA (estrogen responsive gene) and *growth associated protein (gap43*, axonogenesis marker) but had no effect on *cyp19a1a* (estrogen non-responsive gene) or *alpha tubulin 1 (tubal*, synaptogenesis marker). Microarray analysis identified 82 genes significantly changed within 24 hr after ONX plus ICI and 168 genes after ONX plus fadrozole but only 80 overlapped. Of the ONX-inducible genes repressed by fadrozole, *granulin 1*, *stanniocalcin 2* and *sox11a* and *-11b* were verified by QPCR in independent experiments. Results support the view that estrogen modulates gene expression patterns in zebrafish eye after ONX and suggest that circulating estrogen and neuroestrogen may have separate and distinct actions, and/or that ER-dependent and -independent pathways are operative. Knowledge of genes affected by estrogen in a model of successful regeneration provides an entry point for functional analysis.

McCurley AT, Callard GV (2010). Time course analysis of gene expression patterns in zebrafish eye during optic nerve regeneration. *J Exp Neurosci* 4:1-17.

Sources of Research Support: NIEHS (P42 ES07381); NICHD (2T32 HD073897); EPA (NCER/STAR R831301).

Nothing to Disclose: ATM, RKM, PNF, GVC

Pub # P1-357

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Reproductive Experience Alters Neural and Behavioral Responses to Acute Activation of Estrogen Receptor Alpha

Author String RS Bridges, KM Casey, L O'Connell, LM Carini, DF Lovelock, EM Byrnes
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Body Reproductive experience (i.e. parturition and lactation) leads to persistent alterations in anxiety-like behavior that are estrous cycle dependent. We recently found that the administration of the selective ER α agonist PPT results in anxiolytic-like behaviors on the elevated plus maze (EPM) in primiparous female rats. Similar effects were not observed in age-matched, nulliparous controls. To elucidate potential shifts in neural activation underlying these effects, the present study examined induction of the immediate early gene cFOS in multiple brain regions following administration of PPT in primiparous and age-matched, nulliparous female rats tested on the EPM. Significant differences in cFOS immunoreactivity were observed in both the central nucleus of the amygdala (CeA) and the paraventricular nucleus (PVN). As both of these regions contain cell bodies that produce corticotrophin releasing hormone (CRH), a neuropeptide that has been shown to play a role in anxiety-like behavior, further studies investigated changes in CRH gene expression using qPCR, as a function of reproductive experience in both of these brain regions. Significant parity related differences in CRH mRNA within the PVN were observed following administration of PPT. Specifically, PPT reduced CRH gene expression in primiparous females tested on the EPM when compared to similarly treated nulliparous controls. No significant differences in CRH mRNA expression were observed in the CeA. These data indicate that reproductive experience alters the neural response to ER α activation in brain regions that modulate anxiety-like behavior. Further studies are needed to delineate the possible role of CRH in these effects.

Sources of Research Support: NIH Grant HD039895 awarded to RSB.

Nothing to Disclose: RSB, KMC, LO, LMC, DFL, EMB

Pub # P1-358

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Lateral Hypothalamic Leptin Receptor Neurons Containing Neurotensin Regulate Orexin Neurons, Energy Balance and the Mesolimbic Dopamine System

Author String GM Leininger, DM Opland, Y-H Jo, M Faouzi, L Christensen, H Yang, JB Becker, RC Thompson, MG Myers, Jr
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Body Leptin acts via leptin receptor (LepRb)-expressing neurons in the brain to regulate energy balance- in part by modulating the mesolimbic dopamine (DA) system. LepRb neurons in the lateral hypothalamic area (LHA) represent potential mediators for these leptin effects, since the LHA contains cells, including orexin (OX) neurons, that project to the ventral tegmental area (VTA; a core component of the mesolimbic DA system) and that modulate the mesolimbic DA system. We found that the majority of LHA LepRb neurons contain the neuropeptide neurotensin (Nts). Nts-expressing LepRb neurons (LepRbNts neurons) are restricted to the LHA and project to the VTA as well as onto local OX neurons. To investigate the physiological importance of leptin action via LepRbNts neurons, we generated mice null for LepRb specifically in LHA Nts neurons (Nts-LepRbKO mice). Nts-LepRbKO mice demonstrate increased body weight and adiposity, modestly increased feeding, and decreased locomotor activity. Furthermore, the regulation of OX activity and gene expression by fasting and leptin is compromised in Nts-LepRbKO mice, as is the mesolimbic DA system. Overall, our data reveal that LHA LepRbNts neurons mediate important aspects of leptin action on OX neurons and the mesolimbic DA system; disruption of leptin action on this circuit alters DA-dependent behaviors and promotes obesity.

Sources of Research Support: NIH DK57768, DK78056, the ADA (MGM) and NIH DK090101 and TOS (GML).

Nothing to Disclose: GML, DMO, Y-HJ, MF, LC, HY, JBB, RCT, MGM

Pub # P1-359

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Evidence for the Existence of a Local Sympatho-Endocrine System in the Adenohypophysis of Mice: Expression of Catecholamine-Synthesizing Enzymes in Hormone-Producing Cells

Author String I Bodnar, ZE Toth, A Dobolyi, M Olah, GM Nagy
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Body The rate-limiting step of catecholamine biosynthesis is the formation of L-3,4 dihydroxyphenylalanine (L-DOPA) from tyrosine catalyzed by the tyrosine hydroxylase (TH). Phosphorylation of TH at Ser40 (p-TH) in situ is critical for its activation. It must be noted that the dephosphorylated TH is inactive. TH can be found in the central nervous system, as well as in the adrenal medulla and in the sympathetic nervous system. Sporadic observations suggest that TH may be expressed in some endocrine i.e. non-neuronal tissues including the islet cells of pancreas and the anterior lobe (AL) of the pituitary gland. We have recently found that TH immunopositive cells are present in the AL of male CD1 mice. Surprisingly, the same cells can also be labeled with p-TH antibody, indicating that TH is present in its active form, which clearly suggest a local synthesis of L-DOPA, probably dopamine (DA) as well. Performing TH and p-TH immunostaining on dispersed AL cells, 2-3 % of them were labeled. Using in situ hybridization, it has been found that TH mRNA can also be detected in AL cells with the same pattern of distribution like TH and p-TH. Combining in situ hybridization for TH with PRL or TSH immunohistochemistry, no co-localization could be detected. Since DA could be further processed to norepinephrine (NE) by dopamine-beta-hydroxylase (DBH), RT-PCR for DBH mRNA was used to investigate the expression of this enzyme in the pituitary gland. It was shown that substantial amount of DBH mRNA is present in the AL, but not in the neuro-intermediate lobe of the pituitary gland. This result has prompted us to look at the presence of the translated peptide as well. Not surprisingly, DBH immunoreactive cells could also be detected in the AL, but they were more numerous, and they showed different distribution pattern compared to TH positive cells. DBH immunostaining on dispersed AL cells revealed that 10-12 % of them can produce DBH. Treatment of dispersed AL cells with alpha-methyl-p-tyrosine, an inhibitor of TH, significantly reduced ACTH, but had no effect on GH and PRL release. While treatment of cells with FLA63 (a DBH inhibitor) has no effect on the release of any of these three hormones, but in combination with DA, it enhanced PRL and reduced ACTH inhibitory response induced by DA alone. These results indicate that locally formed DA and its conversion to NE can have both positive and negative paracrine influence on hormone release in the AL.

Sources of Research Support: OTKA-81522 and ETT-458/2009 to GMN, ETT 459/2009 and the Bolyai Fellowship to ZE Tóth.

Nothing to Disclose: IB, ZET, AD, MO, GMN

Pub # P1-360

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Role of Clusterin in Neuroendocrine Regulation and Stress Responses

Author String M-s Shin, H Chang, C Namkoong, JY Park, KU Lee, KH Park, M-S Kim
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Body **BACKGROUND:** Clusterin (also called apolipoprotein J) is a sulfated glycoprotein that is implicated in immune regulation, tumorigenesis, neurodegeneration, lipid transport, and pancreatic b-cell regeneration. In the previous study performed by our research group, clusterin is abundantly expressed in the hypothalamic neurons and plays an important role in central regulation of body weight homeostasis. In this study, we aimed to investigate a possible role of clusterin in neuroendocrine regulation and stress responses.
METHODS: Firstly, we investigated clusterin expression in mouse hypothalamus and rat pituitary gland using immunohistochemistry. We next examined whether ICV administration of clusterin affects plasma levels of corticosterone, TSH, LH, FSH, GH, prolactin and catecholamines. We also investigated if ICV administration of clusterin induces anxiety-like behaviors during elevated plus maze test. We finally tested if clusterin deficiency affects stress-induced hormonal and behavioral responses.
RESULTS: Clusterin was abundantly expressed in the multiple hypothalamic areas including the arcuate nucleus and paraventricular nucleus, which play an important role in neuroendocrine regulation. Moreover, clusterin was highly expressed in the intermediate lobe of the pituitary gland with lower expression in the anterior and posterior pituitary glands. In the pituitary glands, clusterin expression was largely overlapped with that of the POMC product, a-MSH in the intermediate lobe but not in the anterior lobe. Administration of clusterin peptide into the cerebroventricle of C57BL/6 mice significantly increased plasma corticosterone and epinephrine level ($P < 0.05$). Furthermore, plasma LH and FSH levels were decreased by ICV injection of clusterin compared to ICV injection of saline ($P < 0.05$). Plasma TSH concentrations were not significantly altered following ICV administration of clusterin. Plasma GH and prolactin levels tended to higher at 20 min post-injection but did not reach a statistical significance. ICV administration of clusterin also increased anxiety-like behaviors in elevated plus maze test ($P < 0.05$). Consistently, clusterin-deficient mice had reduced anxiety-like responses in elevated plus maze test ($P < 0.05$), but displayed normal corticosterone responses following drowning stress.
CONCLUSIONS: Clusterin may be an important player in physiological adaptation of neuroendocrine function and behaviors in stressful condition.

Nothing to Disclose: M-SS, HC, CN, JYP, KUL, KHP, M-SK

Pub # P1-361

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Central Insulin Resistance Impacts the Serotonergic System and Leads to Behavioral Abnormalities with Aging

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Body The ability of neurons to sense and respond to nutrients and hormones is of crucial importance in control of food intake, glucose and lipid metabolism, all of which can play a role in insulin resistance and type 2 diabetes. Rodent and human data have shown that insulin resistance and diabetes may accelerate brain aging and neurological comorbidities such as Alzheimer's disease and depression. Whether central insulin resistance contributes to these neurological abnormalities, however, remains unclear. We have previously shown that mice with a disruption of the insulin receptor in the brain (NIRKO mice) have altered feeding activity and hypothalamic hypogonadism, while having no detectable behavioral abnormalities at a young age. Here we show that NIRKO mice subjected to aging develop significant alterations in neurological function and behavior.

To test behavioral abnormalities, 11 and 17 month old NIRKO mice were used in anxiety and depression activity tests. 17 month old NIRKO mice displayed increased periods of immobility during tail suspension and Porsolt tests in comparison to controls, suggestive of increased depressive-like behavior. This phenomenon was only observed in the oldest cohort and was not apparent with the 11 month old mice. To elucidate anxiety 11 and 17 month old NIRKO mice were used in a modified open field test. Fasted mice were exposed to an open field with centrally placed food. There was no difference in odor perception between the groups, but 17 month old NIRKO mice required more time to enter the center of the field compared to control. In addition, when 17 month old NIRKO mice were exposed to restraint stress, basal, as well as stress-induced, corticosterone levels were increased in NIRKO mice compared to control. Additionally, basal body temperature of NIRKO mice was decreased in both cohorts, and the 17 month old mice displayed an additional blunted response to 2h cold exposure. To understand the cause of these phenotypes, neurotransmitter receptor expression was examined in 3 month old NIRKO mice. Even in these mice, quantitative PCR analysis showed decreased expression of serotonin receptors in the hippocampus of NIRKO mice. Serotonin receptors are known to regulate both body temperature and mood. Thus, taken together these data show the importance of central insulin signaling on the serotonergic system of neurotransmission, which may contribute to altered regulation of body temperature and altered behavior during aging.

Nothing to Disclose: AK, HAF, CRK

Pub # P1-362

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Chronic Estradiol-17 β Exposure Increases Endothelin-1 Activity in the Paraventricular Nucleus

Author String M Subramanian, C Northcott, P Balasubramanian, GD Fink, SMJ MohanKumar, PS MohanKumar
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Body Women are chronically exposed to estrogen through the environment and/or through hormonal therapy. Conventionally, estrogen is believed to be cardioprotective though the Women's Health Initiative studies have questioned this belief. Previous studies from our laboratory have provided evidence that chronic exposure to low levels of estradiol17 β elevates mean arterial pressure in young female Sprague-Dawley (SD) rats. In the present study, we hypothesized that estradiol's effects on mean arterial pressure are mediated through endothelin-1 (ET-1) in the paraventricular nucleus (PVN), a critical area for cardiovascular regulation. To test this hypothesis, young female SD rats were either sham implanted or implanted subcutaneously with 90 day slow-release estradiol17 β pellets (20ng/day; Innovative Research America, FL). At the end of treatment, animals were sacrificed; brains were removed and frozen immediately on dry ice. Trunk blood was collected and the serum was separated to measure estradiol levels by RIA. Brains were sectioned and the PVN was microdissected from serial sections using Palkovit's microdissection technique. Gene expression of ET-1 and ET-1 A and ET-1 B receptors in the PVN were assessed by qPCR. ET-1 A protein levels were assessed using western blot. Serum estradiol levels (mean \pm SE; pg/ml) were significantly higher in estrogen treated animals (68.2 \pm 4.1) compared to the controls (46.7 \pm 2.9; p<0.05). There was a significant increase (p<0.05) in the mRNA levels (mean \pm SE; fold change in 2^{-[Delta][Delta]Ct} values) of ET-1 (1.6 \pm 0.4) and ET-1 A receptor (1.6 \pm 0.1) in the PVN of estrogen treated animals when compared with the sham-implanted controls (0.69 \pm 0.1 and 0.86 \pm 0.2 respectively). There was no change in ET-1 B mRNA between control and estradiol treated animals. Western blot analysis of the PVN showed a marked increase ET-1 A receptor levels (mean \pm SE; densitometric units) in estrogen treated animals (66.5 \pm 6.1) compared to controls (47.1 \pm 3.6) (p<0.05). Our results suggest that increases in endothelin activity in the PVN could possibly play a role in chronic estradiol-induced hypertension.

Sources of Research Support: NIH AG027697.

Nothing to Disclose: MS, CN, PB, GDF, SMJM, PSM

Pub # P1-363

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Neuroendocrine Regulatory Peptides (NERPs) Localize in Human Neurons and Endocrine Tissues

Author String H Yamaguchi, T Matsuo, H Kageyama, K Sasaki, S Shioda, N Minamino, M Nakazato
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Body *Vgf* was originally identified in rat pheochromocytoma PC12 cells as a nerve growth factor-responsive gene. In adult rats, *vgf* is expressed in the brain, spinal cord, subsets of endocrine cells in the pituitary gland, adrenal medulla, pancreas, and gastrointestinal tract. We have identified two novel biologically active peptides, designated neuroendocrine regulatory peptides NERP-1 and NERP-2, that are secreted from human medullary thyroid carcinoma TT cells (J Biol Chem, 282, 26354-60, 2007). NERP-1 (PESA-25, VGF₂₈₁₋₃₀₆) and -2 (QAEA-38, VGF₃₁₀₋₃₄₇) are 25-amino acid and 38-amino acid peptides with C-terminal amidation, respectively, and are abundant in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) in rat hypothalamus. In the PVN and SON, NERPs colocalize frequently with vasopressin (AVP), but rarely with oxytocin. NERPs dose-dependently suppress AVP release induced by intracerebroventricular injection of high salt or angiotensin II (AII) *in vivo*. NERPs also suppress basal and AII-induced AVP secretion from hypothalamic explants *in vitro*. C-terminal amidation of NERPs is essential for biological activity. To explore the localization of NERPs in human tissues, we performed immunohistochemistry analysis on tissues obtained at autopsy or surgery. In the hypothalamus, cell bodies that were stained strongly for NERPs were observed in the vasopressin-rich SON and PVN where vasopressin was abundant. Immunoreactive (ir) NERPs were detected in the islets of the pancreas, where they colocalized extensively with insulin, partially with glucagon, and not at all with somatostatin. Ir-NERPs were also detected in the thyroid and gastric antrum, where they colocalized with calcitonin and gastrin, respectively. NERPs were colocalized with insulin in an insulinoma specimen. Using a specific immunoassay system, NERPs were found to be abundant in the pancreas, with tissue contents of ir-NERP-1 and -2 being 4.5 ± 2.2 and 1.0 ± 0.3 pmol/g wet tissue, respectively. Ir-NERPs of the human pancreas, thyroid and gastric antrum behaved identically to synthetic human NERP-1 or -2 on reverse phase-high performance liquid chromatography combined with radioimmunoassay (Regul Pept, 163, 43-48, 2010). In summary, the presence of NERPs in the human hypothalamus, defined cell types in islets, thyroid C-cells, and gastrin-producing G cells in the stomach antrum suggests that NERPs might function as local modulators in the neuroendocrine system.

Nothing to Disclose: HY, TM, HK, KS, SS, NM, MN

Pub # P1-364

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Increased Responsiveness to Leptin Induced by Estradiol Is Mediated by CRF Type 2 Receptor

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Body Gonadal hormones influence food intake and body weight. We have previously observed that ovariectomized (OVX) rats treated with estradiol cypionate have an increased hypothalamic CRF mRNA. Therefore, the aim of this work was to investigate the role of CRF on food intake, body weight and uncoupling protein-1 (UCP1) content in response to central stimulation with leptin in estradiol-treated OVX rats. Female Wistar rats (200-250g) were subjected to both ovariectomy and cannula implantation into the lateral ventricle. On the next day rats were treated with estradiol cypionate (OVX+E, 10 [micro]g/kg body weight, sc) or vehicle (corn oil: 0.2 mL/rat, sc) for 8 days. Half of OVX rats received diet *ad libitum* (OVX), and half received the amount of food ingested by OVX+E rats (OVX+PF). On the 9th day, food was removed at 4 pm. The animals received icv injections of antisauvagine-30, a specific CRF receptor-2 antagonist (ASG: 5[micro]g/5[micro]L) or vehicle, and 15 min later the rats received icv injection of leptin (10[micro]g/5[micro]L) or saline. Diet was presented 60 min after leptin injection, and food intake and body weight were determined 14h after. Animals were killed and the brown adipose tissue removed to exam UCP1 content by Western blotting. Leptin decreased body weight (OVX: -2.8±0.9g vs 6.0±2.9g; OVX+E: -4.4±3.0g vs 2.2±3.5g; OVX+PF: -0.4±2.7g vs 2.6±4.7g) and food intake (OVX: 4.7±0.5 vs 12.4± 2.1 g/100g body weight; OVX+E: 5.2±0.8 vs 9.2±0.9 g/100g body weight; OVX+PF: 5.5±3.5 vs 7.9±1.3 g/100g body weight) in all groups (p<0.05, n=7), compared to the respective vehicle. The body weight loss after leptin stimulation was higher in the OVX+E group, compared to OVX and OVX+PF groups. Treatment with ASG partially reversed body weight variation and food intake in response to leptin (p<0.05) in OVX+E animals (-2.1±3.7g; 9.7±2.3g/100g body weight, respectively). Estradiol treatment increased UCP1 content (202.3±18.05%, p<0.05, n=5), compared to vehicle treatment. Leptin treatment increased UCP1 content in OVX and OVX+E animals (51.3% and 69.1%, respectively), however, ASG pre-treatment decreased the UCP1 content in response to leptin only in OVX+E rats (p<0.05), compared to vehicle pre-treatment. In conclusion, estradiol increases the responsiveness to leptin in OVX rats and this effect is at least in part mediated by CRF2 receptors.

Nothing to Disclose: PBM, JA-R, LLKE

Pub # P1-365

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Expression of CRH-Related Peptides in the Brain and Heart of Rat Model of Left Ventricular Heart Failure

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Body The roles of neuropeptides such as the CRH family on the condition of heart failure remain unknown. In this study, we analyzed the mRNA expression of CRH-related peptides in the brain, heart and other organs of model rats with left ventricular heart failure. First, we surgically prepared 10 model rats (Ao-banding rats) by banding transverse aorta on mechanical ventilation. Four weeks later, we sacrificed them after assessment of their left ventricular endo-diastolic pressure (EDP) and endo-systolic pressure (ESP) by microcatheterization. Then, we studied mRNA levels of urocortin (Ucn)-1, -2, -3 and Crh in the brain, heart, kidney and adrenal gland by quantitative RT-PCR. Particularly, the brains of both Ao-banding and control rats were sectioned into 9 regions of frontal cortex, striatum, hippocampus, lateral septum, thalamus, hypothalamus, amygdala, pituitary gland and cerebellum. In addition, serum concentrations of BNP, Ucn-1,-2,-3 and corticosterone were measured by EIA kits.

In the Ao-banding rats, serum BNP, Ucn-2 and Ucn-3 levels were significantly elevated compared with control rats. Furthermore, quantitative RT-PCR demonstrated that Ao-banding markedly induced higher expression of Ucn-2 and Ucn-3 mRNAs in the striatum, hippocampus, thalamus, hypothalamus, amygdala, cerebellum, heart, kidney and adrenal gland of experimental rats than in those of controls. On the other hand, Ucn-1 mRNA levels were increased in the thalamus, hypothalamus and amygdala of the Ao-banding rats compared with controls. Crh mRNA levels in the frontal cortex, thalamus and hypothalamus of the Ao-banding rats were higher than in those of controls. However, Ucn-1 and Crh mRNAs were undetectable in the heart, kidney and adrenal gland of both Ao-banding and control rats.

These findings suggest that Ucn-2 and Ucn-3 can be sensitive markers for left cardiac overloading, and furthermore they may mediate some critical aspects of the physiological response to heart failure in the central nervous system as well as in the heart.

Nothing to Disclose: TM, IM, HI, MY, KT

Pub # P1-366

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Effects of Chronic Estrogen Treatment on Brain-Derived Neurotrophic Factor (BDNF) Expression in the Hippocampus -- Implications in Behavioral Disorders

Author String P Balasubramanian, M Subramanian, PS Mohankumar, SMJ Mohankumar
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Body Women are chronically exposed to estrogen through hormonal preparations like oral contraceptives and hormonal replacement therapy. Chronic estrogen treatment is known to produce behavioral changes in female Sprague-Dawley (SD) rats. Decrease in Brain derived neurotrophic factor (BDNF) in the hippocampus has been implicated in the development of anxiety, behavior and memory disorders. Hence, we hypothesized that chronic estrogen treatment causes downregulation of *BDNF* in the hippocampus to cause anxiety like behavior. In order to test this hypothesis, we used adult female SD rats and were either sham-implanted or implanted with 90 day slow release estradiol-17 β pellets (20ng/day). At the end of the treatment, the animals were sacrificed and their brains were collected and snap-frozen on dry ice. Brains were serially sectioned and the hippocampus was microdissected using Palkovit's microdissection technique. The mRNA and protein levels of BDNF were measured in the hippocampus using q-PCR and ELISA. The results demonstrated a significant decrease in the mRNA and protein levels of *BDNF* in the hippocampus of estrogen treated animals when compared to sham-implanted controls. We also wanted to test if the decrease in BDNF gene expression observed in estrogen treated animals is associated with epigenetic changes like hypermethylation in the promoter region of the BDNF gene. To test this, methylation-specific PCR was performed in the bisulfite treated DNA extracted from the hippocampus of estrogen treated and sham implanted animals. Methylation-specific PCR analysis showed that there is a significant upregulation in the methylated-BDNF gene levels in the estrogen treated animals, suggesting hypermethylation of the promoter region of *BDNF* gene in the hippocampus. Our results suggests that epigenetic mechanisms could possibly play a role in chronic estrogen induced anxiety like behaviour.

Sources of Research Support: NIH AG027697.

Nothing to Disclose: PB, MS, PSM, SMJM

Pub # P1-367

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Period Genes Regulate the Metabolic Response to Sleep Loss

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Body Human and animal studies show that sleep has a strong impact on the regulation of metabolic homeostasis. Lack of sleep increases food intake correlating with a deregulation of peripheral appetite-controlling hormones such as leptin or ghrelin. At the same time many metabolically relevant pathways are controlled by a network of circadian clocks that coordinates the synchronization of physiology and behavior to the 24 h rhythm of the Earth rotation around its axis.

To study sleep clock interaction in this context we analyzed the impact of clock gene function on the metabolic response to sleep restriction. We used a sub-chronic gentle handling approach in wild-type and congenic *Per1/2* double mutant mice that lack a functional circadian clock. Mice were sleep deprived for 6 hrs each morning (ZT0-6) on 5 consecutive days. We measured locomotor activity, body weight and food intake as well as circulating hormones and gene expression profiles in adipose tissue and liver before, during and after sleep restriction.

In wild-type animals locomotor and feeding activity patterns as well as adipokine levels were altered after sleep restriction. *Per1/2* mutants showed significant changes in the metabolic effects of sleep restriction at, both, behavioral and endocrine levels. Our findings suggest that circadian clock genes play a functional role in the regulation of metabolic homeostasis by sleep.

Sources of Research Support: German Research Foundation (DFG).

Nothing to Disclose: JH, CH, GE, HL, HO

Pub # P1-368

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Neuroendocrine Regulation of Behavior (1:30 PM-3:30 PM)

Title Sleep, the Circadian Clock, and Metabolism

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Body Most industrialized societies today suffer from an increasing pandemic of obesity at great social and economic cost. Factors contributing to this development include sleep curtailment, increased long distance travel and shift work, all factors previously shown to disrupt circadian timing. Circadian clocks are internal timers that are synchronized by external time cues such as light and nutrient availability, and direct the appropriate physiological and behavioral response to maintain efficient use of resources and, thus, homeostasis. The 'master clock' resides in the suprachiasmatic nuclei (SCN), and is responsible for the co-ordination of peripheral clocks in most organs of the body, including the liver and adipose. It is known that disruption of the circadian clock has metabolic consequences, as well as impacting on sleep regulation. This study aims to establish whether dysregulation of the circadian clock is a major link between sleep curtailment and its metabolic consequences. Following two weeks of partial sleep restriction in mice, a global disruption of transcriptional rhythms was seen in the SCN, liver and adipose tissue. This was correlated with changes in key metabolic parameters, which persist despite resumption of normal activity in the post-sleep restriction phase. This indicates a direct role for the circadian system in the metabolic response to sleep curtailment.

Sources of Research Support: German Research Foundation (DFG).

Nothing to Disclose: JLB, JH, BB, JK, HL, HO

Pub # P1-369

Session Information POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)

Title Sumoylation Componentry and Endocrine Disruptors: Differential Effects on SF-1 and SF-1 Target Genes in α T3-1 and L β T2 Gonadotropes

Author String JJA Lambertucci-Bonnet, SL Wilkinson, A Savage, CA McArdle, IM McGonnell, MB Lee, A Sunters, RC Fowkes
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Body Pituitary gonadotropes require the orphan nuclear receptor, steroidogenic factor 1 (SF-1) for appropriate development and function. SF-1 null mice lack gonadotropins and show reduced gonadotrope numbers, contributing to their severe reproductive phenotype. Recent studies implicate two independent mechanisms through which SF-1 activity can be regulated; post-translational modification by sumoylation, and activation by endocrine disrupting chemicals such as the herbicide Atrazine (ATR). To date, very little is known about sumoylation or ATR in the regulation of gonadotrope SF-1. Expression studies for key components of sumoylation machinery in α T3-1 and L β T2 gonadotropes using RT-PCR, revealed expression of *Sumo1*, *Sumo2*, *Sumo3*, *Piasxa*, *Piasy*, and *Pias1*. To determine whether the key endocrine regulator of gonadotrope activity, GnRH, could alter the expression of sumoylation genes, L β T2 cells were treated with either 0 or 100nM GnRH, chronically or in a pulsatile manner (1x5 min pulse/h) for 4h. There were no obvious changes to the level of the sumoylation genes in response to GnRH, although the expression of *Egr-1* was dramatically increased in response to pulsatile GnRH. In similar experiments, total proteins were extracted and subjected to Western blotting for SF-1 expression. GnRH-treated L β T2 cells showed elevated SF-1 expression, regardless of the treatment paradigm (i.e. chronic or pulsatile). Interestingly, additional protein species, consistent with mono-sumoylated SF-1 were observed in all samples, and the intensity of this band increased following exposure to GnRH for 4h. These same protein extracts were also probed for phosphorylated (pERK) and total ERK1/2 expression. A robust pERK response was observed to pulsatile, but not chronic, GnRH-treated extracts. To establish whether ATR could alter SF-1 and SF-1 target genes in gonadotropes, α T3-1 and L β T2 cells were treated with a range of concentrations of ATR for up to 24h. RT-PCR was performed to determine the expression of gonadotrope markers. Opposing effects of ATR were seen in α T3-1 and L β T2 cells, in that ATR increased *Cga* and enabled *Lh β* expression in α T3-1 cells, yet inhibited expression of both genes in L β T2 cells. The *Egr-1* response to ATR was biphasic, whereas *Sf-1* was enhanced by ATR in both cell lines. These data reveal gonadotrope SF-1 is subject to sumoylation and regulation by endocrine disruptors, and suggest novel mechanisms by which SF-1 activity might be altered in the pituitary.

Sources of Research Support: Wellcome Trust Vacation Studentship.

Nothing to Disclose: JJAL-B, SLW, AS, CAM, IMM, MBL, AS, RCF

Pub # P1-370

Session Information POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)

Title Distinct Signaling Cascades Activated by GnRH Mediate the Differential Control of FSH β Transcription Via Activation of CREB and ICER

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Body Expression of pituitary follicle-stimulating and luteinizing hormones (FSH and LH) under the control of pulsatile gonadotropin-releasing hormone (GnRH) is essential for fertility. FSH and LH contain distinct FSH β and LH β subunits, which are preferentially stimulated at low and high GnRH pulse frequencies respectively. The cAMP response element binding protein (CREB) and inducible cAMP early repressor (ICER) are two transcription factors implicated in the regulation of rat FSH β gene expression. We hypothesized that CREB and ICER are activated by two distinct signaling pathways in response to pulsatile GnRH. Stimulation of the gonadotrope-derived L β T2 cell line with GnRH resulted in a time-dependent increase in phosphorylated CREB (pCREB) levels within 30 min. This response was attenuated by a PKA inhibitor (H89) in a dose-dependent fashion, whereas PKC (GF109203X), CamKII (KN-93) and MEK1/II (U0126) inhibitors had minimal effects, only at high concentrations. In contrast, GnRH induction of ICER mRNA levels was significantly attenuated by the MEK inhibitor. To confirm the effect of PKA inhibition on GnRH-stimulated CREB phosphorylation, experiments using L β T2 cells transfected with a dominant negative PKA expression vector (DNPKA) demonstrated a significant reduction in GnRH-stimulated pCREB levels, compared to empty vector controls. Similarly, DNPKA markedly reduced GnRH stimulation of rat FSH β LUC activity, but had little effect on GnRH-stimulated LH β LUC activity, indicating relative specificity of this pathway for GnRH stimulation of FSH β . In perfusion studies, both FSH β mRNA levels and FSH β LUC activity were increased by pulsatile GnRH, with significantly greater increases at low (every 120 min) compared to high (every 30 min) pulse frequencies. Overexpression of DNPKA greatly reduced GnRH-stimulated FSH β mRNA levels and FSH β LUC activity at both low and high pulse frequencies. Correlating with FSH β activation, levels of pCREB were increased to a greater extent by low compared to high GnRH pulse frequencies. The induction of pCREB was also attenuated by overexpression of DNPKA at both low and high pulse frequencies. In conclusion, it appears that the signaling pathways mediating GnRH activation of CREB and ICER are distinct. The PKA pathway mediates GnRH-stimulated CREB phosphorylation and FSH β transcription in response to continuous as well as pulsatile GnRH at both low and high pulse frequencies, whereas GnRH induction of ICER occurs via MAPK pathways.

Nothing to Disclose: IRT, NAC, SX, AK, RSC, UBK

Pub # P1-371

Session Information POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)

Title The Heptapeptide of LH β Subunit Directs the Exit of Lutropin from the Endoplasmic Reticulum to the Regulated Secretory Pathway in GH3 Cells

Author String A Jablonka-Shariff, I Boime
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Body LH, FSH, TSH and hCG comprise the glycoprotein heterodimer family of a common α subunit and a hormone-specific β subunit. LH and TSH are secreted through the regulated pathway, i.e., stimulated by secretagogues, while FSH and hCG are secreted constitutively. Defining the sorting signals is critical for understanding the link between their secretion and reproductive role. All share an identical α subunit and the β subunit contains the determinants for sorting. One feature of LH β is carboxyl terminal hydrophobic heptapeptide (115Leu-Ser-Gly-Leu-Leu-Phe-Leu121). We demonstrated that this sequence is important for regulated-type secretion of LH from transfected rat somatotrope-derived GH3 cells. Recently we showed that the leucine residue at position 118 is critical for its sorting signaling. We reported earlier that the majority of non-combined LH β , FSH β and TSH β subunits accumulate in the ER and are poorly secreted. However, in cells co-expressing the α subunit, the assembly is quantitative (except for the LH β subunit), and the entire β subunit pool exits the cell as dimer. These data show that the assembled α subunit serves as an [ldquo]escort [rdquo] (chaperone) in shuttling the β subunit to distal compartments in the regulated pathway. Here we examine the sorting characteristics of LH β and LH β L118A subunits and their corresponding dimers using confocal microscopy. To determine if LH is localized to secretory vesicles in the regulated pathway, GH3 and CHO cells were double stained with CG β antiserum (cross reacts with LH β), and an antibody against vesicle-associated membrane protein-2, an integral membrane protein of secretory granules. LH wild-type exhibits a fluorescence of cytoplasmic puncta co-localized to secretory vesicles. The LH β L118A mutant had fewer puncta consistent with reduced cellular accumulation. As expected CHO cells, which lack a defined regulated secretion pathway, did not show co-localized staining. A perinuclear staining pattern is observed for LH β with marked co-localization with the ER marker, protein disulfide isomerase indicating that LH β is primarily retained in the ER. A three fold reduction of cells expressing LH β L118A with this staining pattern is observed. In addition, leucine 118 acts in concert with α subunit for sorting to the regulated pathway since secretion of the free LH β L118A mutant is the same as LH β . These data show that the heptapeptide of LH β subunit directs the exit of LH dimer from the ER to the regulated secretory pathway.

Sources of Research Support: NIH Grant HD061907 awarded to IB.

Nothing to Disclose: AJ-S, IB

Pub # P1-372

Session Information POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)

Title Induction of Mitogen-Activated Protein Kinase Phosphatase 1 (MKP1) by Pulsatile Gonadotropin-Releasing Hormone Stimulation and the Role for Gonadotropin Subunit Expression

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Body **Background** In pituitary gonadotrophs, GnRH induces the expression of the mitogen-activated protein kinase phosphatase 1 (MKP1), an extracellular signal-regulated kinase (ERK) dephosphorylating enzyme. Here we examined MKP1 expression levels following pulsatile GnRH stimulation. **Material and Methods** We used L β T2 cells as a model of gonadotropin producing cells. GnRH pulses were delivered by a set of peristaltic pumps controlled by a time controller and cells were treated with 10 nM pulsatile GnRH at either a high frequency (one pulse, 5 min flow every 30 min) or a low frequency (one pulse, 5 min every 2 h). Induction of MKP1 and activity of ERK were determined by western blotting. We performed luciferase assay to measure the gonadotropin subunit promoter activities. **Results** MKP1 expression increased more prominently following high frequency (every 30 min) GnRH pulse stimulation (7.02 ± 1.47 fold) compared to low frequency (every 120 min) GnRH pulses (2.68 ± 0.09 fold). With high frequency GnRH pulses, MKP1 expression increased by 2.89 ± 0.32 fold 2 h after GnRH pulse initiation (4 GnRH pulses of 5 min). MKP1 expression was not induced following lower frequency GnRH pulses, even when the GnRH concentration was increased. Under high frequency conditions, ERK phosphorylation was observed 10 min after the GnRH pulse and decreased to the basal levels at 30 min. However, ERK dephosphorylation did not occur concurrently with DUSP1 expression. Overexpression of MEKK, a kinase upstream of ERK, increased both LH β and FSH β subunit promoter activity, which were completely inhibited by co-transfection with MKP1-expressing vectors. Serum response element (SRE) promoter activities induced by MEKK were also prevented by MKP1 overexpression, confirming that ERK has an important role in gonadotropin subunit gene expression. Both high and low frequency GnRH pulse stimulation failed to increase LH β and FSH β subunit gonadotropin gene expression upon MKP1 overexpression. **Conclusion** Our study demonstrates that MKP1 is specifically expressed following high frequency GnRH pulses, and this effect may participate in the differential regulation of gonadotropin subunit expression in association with ERK phosphorylation.

Nothing to Disclose: INP, HK, TM, AO, KM

Pub # P1-373

Session Information POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)

Title Opposite Regulation of Gonadotropin Subunit Expression Is Mediated by Gas Pathway-Dependent Autocrine Factor Release in the Pituitary Gonadotropes

Author String SG Choi, SC Sealfon
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Body Gonadotropin releasing hormone receptor (GnRHR) is a Gprotein coupled receptor (GPCR) that mediates signaling activity via coupled heterotrimeric Gproteins. To understand the nature of Gprotein signaling activated by GnRHR, we investigated the downstream signaling activity and gene expression profile under the siRNA mediated knockdown of each Ga subunit of Gproteins in LbT2 gonadotrope cells. Transfection of the siRNA resulted in specific down-regulation of each Ga subunit with knockdown efficiency of 80~90% for mRNA and 60~70% for protein without cross-knockdown. We found that Gas, Gaq/11, and Ga12/13 were involved in the GnRHR mediated signaling where the knockdown of each Gprotein specifically affected the expression of early genes and gonadotropin subunits upon GnRH stimulation. Interestingly Gas oppositely regulated gonadotropin subunit expression. Under Gas knockdown, follicle-stimulating hormone beta (FSHb) mRNA expression was strongly up-regulated while luteinizing hormone beta (LHb) mRNA was markedly down-regulated. In subsequent experiments, we found that FSHb mRNA up-regulation under Gas knockdown was mediated by a released autocrine factor, and the factor was proteinaseK sensitive peptide between 10 kDa and 30 kDa molecular weight. The result from microarray and mass-spectrometry provided a list of candidate autocrine factors specifically regulated under Gas pathway. In short, Gas pathway is crucial for differential gonadotropin subunit expression, and the observed FSHb mRNA up-regulation under Gas knockdown was mediated by the released autocrine factor. Further studies are required to identify the factor among the candidates and characterize the mechanism for the differential gonadotropin expression of Gas pathway.

Sources of Research Support: National Institutes of Health Grant DK46943.

Nothing to Disclose: SGC, SCS

Pub # P1-374

Session Information POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)

Title Pituitary Adenylate Cyclase Activating Peptide (PACAP) Mediates the GnRH Stimulatory Effect on LH Beta Transcription in the Rat

Author String DJ Haisenleder, LL Burger, JC Marshall
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Body Pituitary LH and FSH beta gene expression is regulated by pulsatile GnRH. However, the intracellular pathways that mediate GnRH action on the gonadotrope remain to be fully characterized. cAMP regulates LH and FSH beta promoter activity, and GnRH increases cAMP and stimulates other members of the cAMP/PKA pathway (i.e. CREB, CREM, ICER). PACAP also activates the cAMP pathway, and is present within primary pituitary cells, including gonadotropes. Pulsatile GnRH and PACAP both have similar stimulatory actions on gonadotrope cells (i.e. LH beta, FSH beta and follistatin gene expression). Also, GnRH increases PACAP expression, and it is possible that PACAP acts as a mediator in the GnRH action on the gonadotrope. To address this issue, pituitary cells from adult female rats were cultured for 48h, then given 1nM GnRH (3 pretreatment pulses over 6 hours, followed by a final 10 min GnRH stimulus) +/- a peptide PACAP antagonist (2.5uM). Control groups received vehicle +/- PACAP antagonist (n = 5 per treatment group). LH beta, FSH beta and ICER primary transcripts (PTs) were measured by real time PCR. Results showed that GnRH stimulated 3-3.5 fold increases in LH beta, FSH beta and ICER PTs (p<0.05 vs vehicle controls). The addition of PACAP antagonist abolished the stimulatory effect of GnRH on LH beta and ICER PTs and partially suppressed the effect on FSH beta PT (30% decrease, p<0.05). PACAP antagonist treatment alone had no effect on basal levels of LH beta, FSH beta or ICER PTs. These data support the concept that PACAP acts as an intermediary between GnRH actions and the cAMP pathway in gonadotropes. Further, the findings demonstrate a mechanistic interaction between GnRH and PACAP in transcriptional regulation of LH β , and to a lesser extent FSH beta.

Nothing to Disclose: DJH, LLB, JCM

Pub # P1-375

Session Information POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)

Title Gonadotropin Inhibitory Hormone (GnIH) Secretion into the Hypophyseal Portal System of the Sheep and Its Cognate Receptor on Pituitary Gonadotropes

Author String JT Smith, A Rao, IJ Clarke
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Body GnIH was first identified and isolated in avian species, and there is now strong evidence that a similar factor is operant in mammals as an important regulator of reproduction. Mammalian RFRP-3 (now termed GnIH) is encoded by the RFRP gene, in neurons of the ventral paraventricular nucleus (PVN) and the dorsomedial nucleus (DMN) of the hypothalamus. In sheep, GnIH neurons project to GnRH neurons and to the neurosecretory zone of the median eminence, predicting a role as a secreted neurohormone in the direct regulation of gonadotropes of the anterior pituitary. Consistent with this, GnIH reduces pituitary gonadotropin secretion *in vivo* and *in vitro*. To determine whether GnIH is a secreted neurohormone, we measured its concentration in the hypophysial portal blood. Accordingly, paired portal and jugular blood samples were collected every 10 min over 6 h in 6 anestrous ewes and the plasma prepared for assay. A radio-immunoassay was developed. We generated an antibody against ovine/human GnIH in guinea pigs and we used ¹²⁵I-ovine GnIH as tracer and the same peptide as standard. The GnIH antibody identifies GnIH cells in the PVN and DMN by immunohistochemistry, with no positive cells found in other brain regions and specificity has been confirmed through preadsorption studies. The assay has a sensitivity of 3 pg/ml. Samples were extracted with acidified methanol and GnIH was detected in the portal blood of all ewes (range of 2-15 pg/ml). The secretory pattern of GnIH was pulsatile, with a mean pulse amplitude of 3.2±0.4 pg/ml and a mean pulse interval of 52±6 min. Importantly, GnIH concentrations were virtually undetectable in peripheral blood, with no pulses evident. To determine the cellular target for secreted GnIH, we determined the expression of GnIH receptor (GPR147) mRNA in pituitary cells. Fractions enriched for gonadotropes, somatotropes and lactotropes were obtained from ovine pituitaries by Percoll gradient purification and mRNA was extracted for real-time PCR. Gonadotropes expressed GPR147 mRNA, as did somatotropes and lactotropes. These data from sheep show that GnIH is secreted into hypophysial portal blood and may act, as a negative regulator, on pituitary gonadotropes.

Sources of Research Support: Australian NHMRC.

Nothing to Disclose: JTS, AR, IJC

Pub # P1-376

Session Information POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)

Title The Homeodomain Protein Msx1 Represses the α GSU and GnRH Receptor Genes during Gonadotrope Differentiation

Author String H Xie, BD Cherrington, PL Mellon
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Body Multiple homeodomain transcription factors are crucial for pituitary organogenesis and cellular differentiation. A homeodomain repressor, Msx1, is expressed from the ventral aspect of the developing anterior pituitary and implicated in gonadotrope differentiation. Immunohistochemistry of Msx1- β -galactosidase embryos show that Msx1 gene expression is detected in Rathke's pouch starting at e10.5 and at the rostral edge at e13.5 where the gonadotrope-thyrotrope lineage emerges. The subsequent decline in Msx1 expression specifically coincides with the onset of expression of lineage-specific pituitary genes, the common α -subunit (α GSU) and GnRH receptor (GnRHR). Msx1 represses transcription of both the α GSU and GnRHR promoters but not the LH β or FSH β promoters in the gonadotrope-derived alphaT3-1 cell line. In the mouse GnRHR promoter, repression by Msx1 is mediated through a consensus Msx1 binding motif in the downstream activin responsive element (DARE), which binds Msx1 in vitro as determined by EMSA. The human α GSU promoter is also repressed by Msx1 and truncations map this regulation to within -224 bp of the mRNA start site. Two Msx1 binding motifs (-147 and -114) were mapped and both sites bind the Msx1 protein in EMSA. The 147 Msx1 site overlaps with the crucial CRE element, while the -114 Msx1 site overlaps a known Dlx binding site. siRNA knockdown of Msx1 in α T3-1 cells induces an increase in endogenous α GSU mRNA expression further confirming a role for Msx1 in repression of the α GSU. In summary, Msx1 functions as a negative regulator in early pituitary development by repressing the gonadotrope-specific α GSU and GnRHR genes, but a temporal decline in Msx1 expression alleviates this repression allowing induction of GnRHR and α GSU, thus serving to time the onset of gonadotrope-specific gene program.

Sources of Research Support: R01 HD020377, U54 HD012303, R01 DK044838, T32 DK007044, HD007203

Nothing to Disclose: HX, BDC, PLM

Pub # P1-377

Session Information POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)

Title The Effect of GNRH-(1-5) on LH Release

Author String DO Larco, E Pagano, TJ Wu
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Body The decapeptide gonadotropin-releasing hormone (GnRH) is a central regulator of reproduction. After its secretion, GnRH is processed by the zinc metalloendopeptidase EC3.4.24.15 (EP24.15), which cleaves the bond between the 5th and 6th amino acid to form the biologically-active pentapeptide, GnRH-(1-5). We have previously shown GnRH-(1-5) facilitates lordosis behavior in female Sprague-Dawley rats (1). Our *in vitro* studies suggest that the mechanism of GnRH-(1-5) is Ca²⁺-dependent, involving activation of calmodulin-dependent kinase II (CaMKII) (2). In the present study, we examined GnRH-(1-5)-induced phosphorylation of CaMKII (T286) in hypothalamic sites thought to regulate lordosis behavior, including the ventromedial nucleus (VMN) and the anteroventral periventricular nucleus (AVPV). Furthermore, we were interested in whether GnRH-(1-5) regulates the secretion of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH); and if so, whether GnRH-(1-5) alters the structure of the median eminence (ME) as a mechanism explaining its potential role in the modulation of LH and FSH levels. Adult Sprague-dawley rats were ovariectomized and an indwelling cannula was implanted targeting the third ventricle (3V) of the hypothalamus. Subsequently, all animals were randomly divided into 4 treatment groups (vehicle, progesterone, GnRH, and GnRH-(1-5)). Animals were treated with 2ug of estradiol-benzoate 48 hours prior to intracerebroventricular (icv) injection with a treatment. Western blot analysis revealed GnRH-(1-5) treatment significantly increased ($p < 0.05$) pCaMKII in the VMN and in the AVPV ($p = 0.06$) relative to all other treatments. Furthermore, these animals had significantly ($p < 0.05$) higher levels of peripheral LH, but not FSH. Our preliminary analysis of the ME indicates vimentin, a structural protein expressed in tanycytes, is up-regulated with GnRH-(1-5) treatment, indicating that GnRH-(1-5) could be enhancing the release of GnRH into the vasculature. In conclusion, the results of the present study suggest that the mechanism GnRH-(1-5) to facilitate lordosis behavior acts through the CaMKII pathway. Our data also suggests that GnRH-(1-5) modulates LH levels by altering the architecture of the ME.

(1) Wu TJ et al., Endocrinology 2006; 147(5):2544-9

(2) Wu TJ et al. Endocrinology 2005;146(1):280-6

Sources of Research Support: NSF Grant IOS 0544150.

Nothing to Disclose: DOL, EP, TJW

Pub # P1-378

Session Information POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)

Title FoxO1 Inhibits Synthesis of Luteinizing Hormone Beta in Pituitary Gonadotrope Cells

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Body Fertility depends on the production of LH and FSH from gonadotrope cells in the anterior pituitary. Synthesis of these hormones is tightly controlled during the menstrual cycle by a complex network of hormonal signaling pathways that are regulated, in part, by metabolic cues such as insulin. One group of candidate gene that may be regulated by insulin in gonadotropes is the FoxO subfamily of forkhead transcription factors. FoxOs regulate diverse cellular functions, such as apoptosis, stress resistance and metabolism, but their role in the neuroendocrine regulation of reproduction has not been extensively explored. In the present study, we investigate whether FoxO proteins regulate LH gene expression and explore mechanisms of action. We demonstrate that FoxO1 is expressed in mouse gonadotrope cells and that FoxO1 phosphorylation on Ser-256 is regulated by insulin in a PI3K-dependent manner. In addition, FoxO1 cellular localization is distributed between the cytoplasm and nucleus in cultured gonadotrope cells under serum-free conditions while treatment with insulin or serum increases FoxO1 localization in the cytoplasm. Our studies also show that FoxO1 represses basal transcription and GnRH induction of the LH β promoter in L β T2 cells. Deletion of the FoxO1 DNA-binding domain relieves the suppression, suggesting that the effect of FoxO1 on both basal transcription and GnRH induction of the LH β promoter requires direct DNA binding. Moreover, the effect maps to the proximal region of the LH β promoter that contains binding sites for basal transcription factors such as SF-1 and Ptx-1 as well as the GnRH-induced factor, Egr-1. Interestingly, FoxO1 also blocks induction of LH β promoter with overexpressed Egr-1, indicating that FoxO1 does not require the endogenous Egr-1 promoter to mediate its suppressive effect. Additional experiments are underway to further define the cis regulatory regions on the LH β promoter necessary for FoxO1 suppression and to explore how interactions among FoxO1 and other transcription factors contribute to the suppression of LH β gene expression. In summary, we clearly demonstrate that FoxO1 is regulated by insulin signaling in gonadotropes and that FoxO1 can suppress basal transcription and GnRH induction of the LH β gene. These data also suggest that FoxO1 may play an important role in controlling LH levels in response to metabolic cues such as insulin.

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Nothing to Disclose: DJA, SLM, VGT

Pub # P1-379

Session Information POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)

Title Molecular & Functional Characterization of an Endocannabinoid System in L β T2 Gonadotropes

Author String L Smith, ER Davies, IR Thompson, JJ Lambertucci-Bonnet, A Gastaldello, M Korbonits, M Campanella, RC Fowkes
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Body Cannabinoids are known to exert effects throughout the endocrine system, including in the anterior pituitary. Whilst cannabinoid receptors (CB1 in particular) are expressed in numerous tissues, expression and function of an endocannabinoid system within specific anterior pituitary cell types has not been fully elucidated. We have used well-characterised gonadotroph (L β T2 and α T3-1) and somatolactotroph (GH3) cell lines to establish the expression and functional role for cannabinoid signaling. Initial RT-PCR studies established that L β T2 cells expressed Cnr1, as well as the cannabinoid synthetic enzymes, Faah, Mgl1 and Napepld. α T3-1 and GH3 cells, and primary mouse pituitary tissue only expressed some of these components. Having established that L β T2 cells expressed a complete endocannabinoid system, functional studies were undertaken. As the CB1 (Cnr1) receptor couples to G α i in many tissues, the potential inhibitory effect of CB1 signaling was determined in L β T2 cells. Following transient transfection with reporter genes expression either the human α GSU promoter, the murine Egr-1 promoter, or a Gal4CREB two-hybrid system, cells were stimulated with either 0 or 10nM Forskolin (FSK, adenylyl cyclase activator) in the absence or presence of a range of concentrations of HU210 (a CB1 agonist). HU210 caused an inhibition of the α GSU and Gal4CREB response to FSK of at least 50%, but failed to alter Egr-1 promoter activity. However, HU210 inhibited PMA-stimulated α T3-1 cells, transfected with the Egr-1 promoter. Similar studies in GH3 cells revealed dramatic inhibition in FSK-stimulated α GSU promoter activity in the presence of HU210, suggesting that CB1 couples to G α i in both L β T2 and GH3 cells. As CB1 receptors can couple to numerous G-protein α -subunits, dynamic confocal imaging of intracellular calcium concentrations ([Ca²⁺]_i) was performed, following Fluo4A/M loading. HU210 caused a rapid increase in ([Ca²⁺]_i) in L β T2 cells, suggesting potential coupling to G α q/11. Collectively, these data reveal gonadotrophs and somatolactotroph lineage cells to be putative targets of (endo)cannabinoid signalling in the pituitary, which may reveal potential therapeutic benefits for fertility and growth disorders.

Nothing to Disclose: LS, ERD, IRT, JJAL-B, AG, MK, MC, RCF

Pub # P1-380

Session Information POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)

Title GnRH Stimulates the Expression of Both Subunits of Follicle-Stimulating Hormone Via Distinct Actions of Calcineurin

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Body We have previously reported that GnRH activates the phosphatase calcineurin, and in this study we investigated its role in directing transcription of the follicle stimulating-hormone (FSH) subunit genes. Calcineurin over-expression induced promoter activity of the common α GSU but not the FSH β subunit gene, although its inactivation abolished the GnRH effect on both genes. On the α GSU, we mapped the calcineurin responsive region to 80 bp encompassing the previously reported GnRH-response element which contains the binding site for the calcineurin target, NFAT. We show that GnRH induces nuclear import of NFAT3 in a calcineurin-dependent manner. NFAT3 was detected at the α GSU promoter only after GnRH treatment, and its knock-down substantially reduced GnRH-stimulated α GSU promoter activity. NFAT3 was also found at the promoter of Nur77, and its knock-down significantly reduced GnRH-induced Nur77 transcription and reduced also FSH β promoter activity. We have shown previously that Nur77 plays a role in de-repression of FSH β gene expression in α T3-1 cells, but in L β T2 cells its over-expression alone had no effect on the FSH β promoter. However, when co-transfected with Transducer of Regulated CREB activity (TORC), a strong synergistic effect was seen, and the addition of CREB increased this synergy further. Interestingly however, TORC knock-down appeared to have only a minor impact on the GnRH effect, likely due to GnRH-induced phosphorylation of CREB at S133 which allows recruitment of CBP, but prevents association of TORC with the FSH β promoter. In contrast, TORC is essential for the GnRH-effect on the α GSU transcription and its over-expression activates promoter activity, but this appears to be independent of CREB. As a result of dephosphorylation by calcineurin, we expected GnRH to induce nuclear accumulation of TORC, but this did not occur and TORC appeared to shuttle between the cytoplasm and nucleus constantly. However GnRH did induce changes in the TORC protein, inducing an initial degradation of TORC, much of which is already N-terminally truncated, and facilitating an increase in levels of intact TORC. As the N-terminus of TORC was previously shown to interact with various transcription factors, this likely represents a means of TORC activation, through a mechanism that has yet to be elucidated but appears to involve calpain cleavage and the proteasome.

Nothing to Disclose: PM, LP, ML, SW, ZN

Pub # P1-381

Session Information POSTER SESSION: BASIC - Gonadotroph Biology (1:30 PM-3:30 PM)

Title Role of β -Catenin in FSH β Gene Regulation in L β T2 Gonadotrope Cell Line

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Body β -catenin, the key effector in the canonical Wnt signaling pathway, has been reported to be induced by GnRH exposure in both GnRHR-expressing HEK293 and L β T2 cells in a time-dependent manner (1). By using specific and efficient siRNA against β -catenin in L β T2 cells, we obtain 90% reduction in β -catenin expression. RNA interference studies showed that endogenous basal and GnRH-induced FSH β gene expression was reduced by β -catenin siRNA knockdown in L β T2 cells. Promoter-reporter assays using a 2kb mouse FSH β promoter-luciferase construct also showed that β -catenin knockdown can attenuate FSH β promoter activity. Promoter sequence analysis indicated that there are six potential LEF/TCF binding sites. Elimination of three of these sites located at -1.5kb, -1kb and -976bp in mouse FSH β proximal promoter region did not affect the response to β -catenin knockdown. Bioinformatic network analysis suggested several regulatory targets, including Pitx2 and SMAD4, which might mediate indirect FSH β regulation by β -catenin. C-jun, a transcription factor required for FSH β gene expression, which has been reported to be regulated by β -catenin (2), was not affected by β -catenin siRNA knockdown in our experiment, indicating that c-jun might not be the major player in β -catenin-mediated FSH β gene regulation. Our data indicate that β -catenin regulates FSH β gene expression. Whether the effect occurs directly at more proximal putative interaction sites or indirectly through other transcription factors is currently under study.

(1) Gardner S et al. Molecular Endocrinology 2007; 21,3028-3038
(2) Salisbury TB et al. Molecular Endocrinology 2009; 23,402-411

Nothing to Disclose: QW, SCS