Insulin and Leptin as Adiposity Signals

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ABSTRACT

There is now considerable consensus that the adipocyte hormone leptin and the pancreatic hormone insulin are important regulators of food intake and energy balance. Leptin and insulin fulfill many of the requirements to be putative adiposity signals to the brain. Plasma leptin and insulin levels are positively correlated with body weight and with adipose mass in particular. Furthermore, both leptin and insulin enter the brain from the plasma. The brain expresses both insulin and leptin receptors in areas important in the control of food intake and energy balance. Consistent with their roles as adiposity signals, exogenous leptin and insulin both reduce food intake when administered locally into the brain in a number of species under different experimental paradigms. Additionally, central administration of insulin antibodies increases food intake and body weight. Recent studies have demonstrated that both insulin and leptin have additive effects when administered simultaneously. Finally, we recently have demonstrated that leptin and insulin share downstream neuropeptide signaling pathways. Hence, insulin and leptin provide important negative feedback signals to the central nervous system, proportional to peripheral energy stores and coupled with catabolic circuits.

I. Overview

When maintained on an *ad libitum* diet, most animals — including humans — are able to precisely match caloric intake with caloric expenditure, resulting in relatively stable energy stores as adipose tissue (Kennedy, 1953; Keesey, 1986). Growing emphasis has been placed on the role of the central nervous system (CNS) in controlling this precision of energy homeostasis. However, to balance the energy equation, the brain must be able to receive several kinds of input from the periphery. Some of these messages should provide information about the status of peripheral energy stores in the form of the adipose mass.

Compelling evidence implicates at least two peripheral hormones as providing key afferent information to the CNS concerning the amount and distribution of body fat. Leptin, a recently described peptide hormone secreted from adipocytes in proportion to fat mass (and especially to subcutaneous fat mass) (Masuzaki *et al.*, 1995; Dua *et al.*, 1996; Montague *et al.*, 1997) has received tremendous attention in recent years. Considerable evidence suggests that leptin acts as one of the body's adiposity signals (Zhang *et al.*, 1994; Matson *et al.*,

1996; Friedman, 1997; Buchanan *et al.*, 1998; Woods *et al.*, 1998; Schwartz *et al.*, 2000). Leptin levels in the blood are correlated with body fat. Administration of exogenous leptin reduces food intake and increases energy expenditure. Furthermore, when energy balance is suddenly changed (for example, if an individual is fasted for a day), plasma leptin levels decrease far more than body adiposity in the short term (Woods *et al.*, 1997; Buchanan *et al.*, 1998). Hence, although much has been written about leptin as an adiposity signal, it is not ideal in and of itself, suggesting that at least one additional signal must exist. The logical candidate is the pancreatic hormone, insulin.

Plasma insulin levels are directly correlated with adiposity (Woods *et al.*, 1998). They correlate better with visceral than subcutaneous fat. Moreover, when energy balance changes, plasma insulin follows these changes faithfully. Hence, leptin and insulin together provide information to the brain not only about the size of the fat mass but also about its distribution and important recent changes of metabolic status. The effort of many laboratories, including part of our own, is concentrated on elucidating how insulin and leptin interact with central neural systems controlling energy homeostasis.

II. Insulin as an Adiposity Signal

Considerable evidence suggests that insulin is a key peripheral regulator of food intake and body adiposity. After Kennedy (1953) hypothesized that fat stores produce a hormone that acts as a negative feedback control for adiposity, one early suggestion was that this signal was insulin (Baskin *et al.*, 1987; Woods *et al.*, 1996). Data supporting this possibility have been collected over the past three decades and include studies using several species and techniques (Woods *et al.*, 2003). To summarize that literature, levels of plasma insulin correlate directly with body weight and with body adiposity in particular (Bagdade *et al.*, 1967; Polonsky *et al.*, 1988a,b). Obese animals and humans have higher basal insulin levels and secrete more insulin in response to a meal than do lean individuals (Bagdade *et al.*, 1967; Woods *et al.*, 1974). Plasma insulin levels also reflect more-acute changes in energy status. Insulin increases during meals and any other condition of positive energy balance and decreases during fasting and other periods of negative energy balance. The major stimulant of insulin secretion is an increase of local glucose levels in the pancreas. The degree of glucose-stimulated insulin secretion is a direct function of body fat (Bagdade *et al.*, 1967; Woods *et al.*, 1974; Polonsky *et al.*, 1988a,b). The increment of insulin in response to glucose lasts only as long as glucose is elevated. Then, insulin is rapidly cleared from the blood, since it has a half-life of only 2 to 3 minutes. Obese animals and humans are said to be insulin resistant because more insulin is required to maintain a normal level of blood glucose. This is important because

insulin resistance and visceral fat correlate with levels of insulin, type 2 diabetes mellitus, and obesity.

The second line of evidence consistent with insulin being a prime candidate for an adiposity signal is the finding that insulin better maps acute changes of energy metabolism and adiposity than its adipocyte cousin leptin. Insulin secretion faithfully tracks changes of energy balance on the order of minutes to hours, as opposed to days, and these changes are always in direct proportion to the size of the adipose mass. Furthermore, insulin plays a key role in the regulation of glucose and lipid utilization and storage. Without sufficient insulin, most tissues cannot take up much glucose, so glucose accumulates in the blood. At the same time, adipocytes cannot take up and store fat. When insulin resistance occurs in obesity, and more insulin is secreted to regulate glucose, the excess insulin causes increased accumulation of fat in adipocytes. Hence, disruptions of insulin sensitivity are associated with both obesity and diabetes. Many reviews of these phenomena have been written (Schwartz *et al.*, 1992; Porte *et al.*, 1998; Woods *et al.*, 1998,2003).

Additional support for the hypothesis that insulin acts as an adiposity signal includes the fact that insulin receptors and insulin receptor mRNA are found in CNS regions involved in the regulation of food intake and body weight (Schwartz *et al.*, 1992; Campfield *et al.*, 1996; Matson *et al.*, 1996; Woods *et al.*, 2003). In particular, insulin receptors in the arcuate nucleus of the hypothalamus (ARC) are prime candidates for translators of an adiposity signal. Third ventricular (i3vt) administration of insulin decreases expression of the anabolic effector peptide, neuropeptide Y (NPY) in the ARC (Schwartz *et al.*, 1992; Sipols *et al.*, 1995). ARC NPY fibers project to the paraventricular nucleus (PVN) of the hypothalamus. Administration of insulin into the i3vt of the brain also causes increased expression of corticotropin (ACTH)-releasing hormone mRNA in the PVN (Sipols *et al.*, 1995; Schwartz *et al.*, 1996b). These results demonstrate that the insulin signal is tied to changes in food intake associated with hypothalamic neuropeptides. The mediation of insulin's catabolic effects is detailed below.

One requirement of a humoral adiposity signal is that it should gain access to the brain. Consistent with this, insulin has been found to enter the brain via a saturable transport process that moves it from plasma into brain interstitial fluid (Hachiya *et al.*, 1988). The rate of entry of insulin to CNS is well mapped to normal fluctuations of plasma insulin levels, although higher levels exceed the saturation point (Baura *et al.*, 1993; Woods *et al.*, 2003). With very high plasma levels, the entry of insulin to brain remains relatively constant. This is important for regulation of body weight because some types of obesity are known to be associated with disruptions of the insulin transport system. A review of this is found in Woods *et al.* (2003).

Finally, and importantly, administration of exogenous insulin into the brain reduces food intake and increases energy expenditure, consistent with its being

an adiposity signal. Repeated administration of insulin in small doses, or as a continuous infusion, elicits decreased food intake and increased energy expenditure after several hours and lasts for the duration of the treatment (Woods *et al.*, 1974,1996; Air *et al.*, 2002a). Administration of insulin into the brain also potentiates the anorexic effects of peripherally administered cholecystokinin (CCK) (Figlewicz *et al.*, 1995; Riedy *et al.*, 1995). This suggests that insulin modulates the body's response to short-term signals that terminate meals. Importantly, administration of insulin peripherally, in amounts that do not cause hypoglycemia, decreases food intake (Nicolaidis and Rowland, 1976; Vanderweele *et al.*, 1982; McGowan *et al.*, 1990; Woods *et al.*, 2003). In agreement with these data, administration of antibodies to insulin into the brain increases food intake and body weight (Strubbe and Mein, 1977; McGowan *et al.*, 1992). It is important to note that there is no evidence that alterations in food intake after administration of insulin, either systemic or central, are secondary to aversive consequences (Chavez *et al.*, 1995). That is, administration of exogenous insulin into the brain does not appear to make animals ill. Collectively, these data suggest that insulin provides a signal of adiposity to the CNS and that the signal is capable of altering food intake, based on the state of energy balance.

In summary, insulin, aside from its important peripheral action for the metabolism of fuels, satisfies all of the criteria for consideration as a general adiposity signal. Indeed, Woods and colleagues (Porte *et al.*, 1998; Woods *et al.*, 1998) have suggested that the administration of insulin does not simply act to change food intake *per se* but rather helps determine the level of fat that will be maintained and defended by the animal. That is, levels of circulating insulin help modulate the long-term level of fat stored in the body in any particular environment. It is unclear at present whether, in the obese insulin-resistant state, there are parallel changes of central insulin-sensitive systems. Niswender and Schwartz (2003) published an excellent recent review of insulin as an adiposity signal.

III. Leptin as an Adiposity Signal

First described in 1994 (Zhang *et al.*, 1994), leptin has proven to be a key metabolic protein that has actions throughout the body. Leptin quickly overshadowed insulin as the best-known adiposity hormone because it is secreted from adipocytes themselves. However, unlike insulin, leptin can be given systemically with few adverse side effects such as hypoglycemia. Analogous to what occurs with insulin, plasma leptin levels are correlated directly with adiposity. Likewise, circulating leptin is transported into the brain via a saturable process (Banks *et al.*, 1996; Schwartz *et al.*, 1996a). Leptin receptors exist in many brain areas, including the ARC (Banks *et al.*, 1996; Schwartz *et al.*, 1996b). Within the brain, leptin has many actions, including reducing food intake and increasing energy

expenditure. This is supported further by the finding that prolonged administration causes loss of body fat and body weight. A current hypothesis is that leptin, being secreted in proportion to total body adiposity, conveys the overall nutritional status to the brain (Ahima *et al.*, 2000). Low leptin levels are considered to indicate depleted fat stores and reduce or turn off functions that require adequate energy stores to be successful (e.g., reproduction). During weight loss, plasma leptin decreases (Boden *et al.*, 1996; Havel *et al.*, 1996; Ahren *et al.*, 1997; Keim *et al.*, 1998); analogously, weight gain is associated with an increase in leptin secretion (Seeley *et al.*, 1996; Ahren *et al.*, 1997).

The importance of leptin as an adiposity signal to the brain is supported further by the phenotype of animals that either do not synthesize it (*ob/ob* mice that have a mutation in the leptin gene) (Zhang *et al.*, 1994) or that have genetic mutations that compromise functioning of the leptin receptor (*db/db* mice and fatty Zucker *fa/fa* rats) (Chua *et al.*, 1996). These animals are characterized by hyperphagia and extreme obesity. Administering small amounts of leptin into the brains of *ob/ob* mice reverses this syndrome (see reviews in Tartaglia *et al.*, 1995 and Woods *et al.*, 1998).

The leptin receptor, a member of the cytokine receptor family, exists in many natural forms in the brain and rest of the body. The leptin receptor can be considered to have two functional parts. The extracellular domain recognizes and interacts with extracellular leptin and is identical for all known leptin receptors. When appropriately activated, the intracellular domain causes certain cellular events to be initiated. Variations in the length of the intracellular domain determine the type of action leptin exerts on a cell (Tartaglia *et al.*, 1995). All intracellular forms of the leptin receptor activate the Janus kinases (JAK) (JAK-signal transducer and activator of transcription (STAT) pathway of tyrosine protein kinases). The form found in the hypothalamus, including the ARC, is the longest one, termed OB-Rb, and has the capacity to activate several intracellular signaling pathways besides JAK-STAT. This form also activates transcription factors (Niswender and Schwartz, 2003).

Leptin signaling (actually, reduced leptin signaling) has been hypothesized to regulate many vital systems when animals are severely hypocaloric and have low body fat. Leptin is secreted from adipocytes in direct proportion to the amount of stored fat, especially the amount of subcutaneous fat. Leptin secretion rate and leptin mRNA expression are two to three times higher in subcutaneous than visceral fat, in part due to the larger adipocytes relative to visceral fat (Dua *et al.*, 1996; Montague *et al.*, 1997; Bray and Popkin, 1998; Samaras *et al.*, 1998). However, the actual stimulus is related more to the metabolic activity of the fat cell than to fat storage, such that dissociations can occur between stored fat and leptin release, particularly during a fast. Nonetheless, under normal conditions, plasma leptin levels are a reliable and rather stable indicator of body fat, since leptin's half-life is about 45 minutes.

Soon after the discovery of leptin, it was reported that when mice are administered exogenous leptin into the brain, they reduce their food intake and body weight (Campfield *et al.*, 1995). Our laboratory replicated this phenomenon in rats (Seeley *et al.*, 1996) and found that leptin exerts this effect without causing signs of illness (Thiele *et al.*, 1997). We also mapped the location of OB-Rb in the brain and determined where leptin administration elicits c-Fos expression (Thiele *et al.*, 1997; van Dijk *et al.*, 1999). We also found that leptin acts in the ARC to reduce NPY synthesis and to stimulate pro-opiomelanocortin (POMC) synthesis (Seeley *et al.*, 1997; Hagan *et al.*, 1999; van Dijk *et al.*, 1999). We proposed a model based upon the opposing actions of anabolic neuropeptides such as NPY and agouti-related peptide (AgRP) and catabolic neuropeptides such as alpha-melanocyte stimulating hormone (α-MSH) (Schwartz *et al.*, 1997). We also reported that the ability of leptin to reduce food intake critically depends upon "downstream" stimulation of activity at melanocortin-4 (MC-4) receptors, since the administration of mixed MC3/4 antagonists or specific MC-4 antagonists attenuates leptin's ability to reduce food intake and induce fos activity in the PVN (Seeley *et al.*, 1997). Like others, we found that the administration of MC-4 agonists reduces food intake and body weight (Thiele *et al.*, 1998; Benoit *et al.*, 2000; McMinn *et al.*, 2000).

IV. Similarities and Differences Among Adiposity Signals

It is worth considering why there should be more than one adiposity signal to the brain. The simple answer is that redundancy is the rule rather than the exception in the control of energy homeostasis. Nonetheless, insulin reflects different fat stores, genders, and risk factors for developing type 2 diabetes mellitus and various cardiovascular problems than does leptin. That is, whereas the levels of insulin and leptin both signal the degree of adiposity to the brain, each has important other functions throughout the body. Insulin is a major controller of the levels and utilization of glucose throughout most of the body. Low circulating leptin and the resultant decrease of leptin signaling have been hypothesized to regulate many vital systems when animals are severely hypocaloric and have low body fat.

There are other fundamental differences. For one, the secretion of insulin is adjusted in response to every acute change of metabolism. Its levels increase during meals or when glucose is elevated for some other reason and decrease during stress and exercise. The half-life of insulin in the blood (2–3 minutes) is consistent with its role as a minute-to-minute indicator of ongoing metabolism; all of its fluctuations are directly proportional to total body fat. Leptin is secreted from adipocytes in direct proportion to ongoing metabolic activity of the fat cell. Plasma leptin levels are, therefore, a reliable and rather stable indicator of body fat. Hence, insulin levels reflect the interaction of ongoing metabolic processes

and body adiposity, whereas leptin levels reflect the activity of adipose cells more directly.

Another difference is that insulin secretion reflects the amount of visceral white adipose tissue, whereas leptin secretion reflects total fat mass, especially subcutaneous fat (Masuzaki *et al.*, 1995; Dua *et al.*, 1996; Montague *et al.*, 1997). This is potentially quite important with regard to the message conveyed to the brain, since visceral fat carries a greater risk for the metabolic complications associated with obesity than does subcutaneous fat. Elevated visceral fat is associated with an increased incidence of insulin resistance, type 2 diabetes mellitus, hypertension, cardiovascular disease, and certain cancers. Hence, while circulating levels of leptin and insulin each convey specific information as to the distribution of fat, the combination of the two conveys information about the total fat mass of the body.

Regardless of the differences, leptin and insulin both provide important afferent information to the brain. Even though they are not made in the brain, the brain nonetheless contains specific receptors for both. Each is transported from the blood, through the blood-brain barrier, by a receptor-mediated mechanism (Porte *et al.*, 1998; Woods *et al.*, 1998,2003). Therefore, biologically active leptin and insulin are delivered into the brain interstitial fluid, where they can interact with receptors on neurons. The ARC contains receptors for each in particularly high concentrations (Banks *et al.*, 1996; Schwartz *et al.*, 1996b; Woods *et al.*, 2003) and each signal has important shared mediators in the hypothalamus. We have found that when insulin and leptin are administered into the brain in combination, they initially interfere with each other's action such that the net catabolic effect is less than the sum of the individual effects. However, after 4 hours, the two peptides are simply additive (Air *et al.*, 2002b). There are several reviews of this literature (Porte *et al.*, 1998; Woods *et al.*, 1998). A diagram of stimulation of the ARC by leptin and insulin is depicted in Figure 1.

V. Central Effector Systems

Signals indicating adiposity, as well as those indicating ongoing metabolic processes and what is being eaten and processed in the gut, converge on the CNS. Within the CNS, in order to regulate food intake and body weight effectively, these signals need to interact in meaningful ways and to engage neurochemical systems that influence energy intake and energy expenditure. The best known of these CNS systems are in the ventral hypothalamus. They can be roughly divided into those whose activity reduces body fat (catabolic effector systems) and those whose activity increases body fat (anabolic effector systems). Anabolic effectors elicit increased food intake, decreased energy expenditure, and consequently increased stored energy in the form of adipose tissue. They are hypothesized to

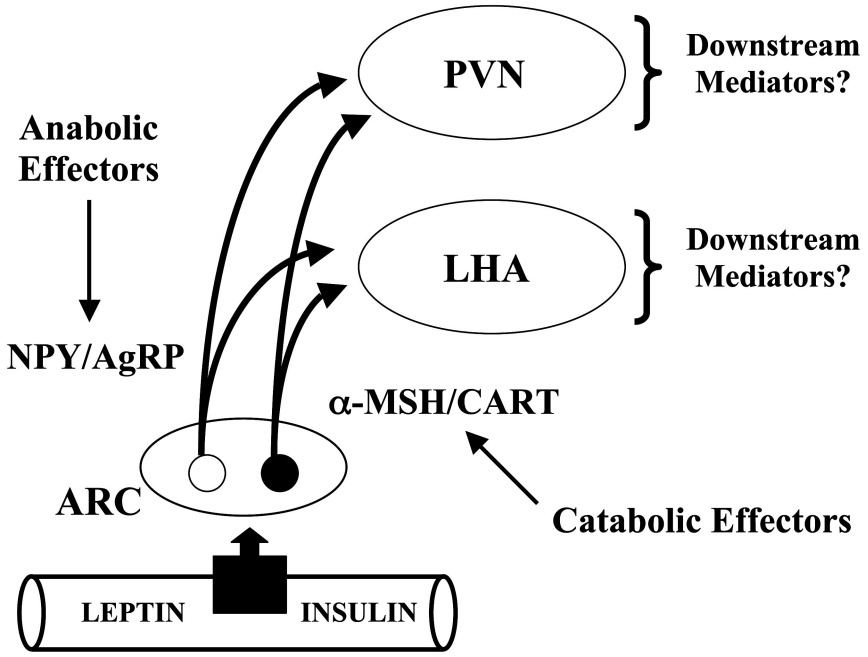

FIG. 1. A diagram representing stimulation of the arcuate nucleus of the hypothalamus (ARC) by leptin and insulin. A neuroanatomical schematic of the hypothalamic melanocortin system control of food intake. Abbreviations: PVN, paraventricular nucleus; LHA, lateral hypothalamic area; NPY, neuropeptide Y; AgRP, agouti-related protein; MSH, melanocyte-stimulating hormone; CART, cocaine- and amphetamine-regulated transcript.

become more active when energy stores are low, as indicated by reduced levels of insulin and leptin (i.e., when the body is in negative energy balance). Catabolic effectors do just the opposite. Activated by positive energy balance, they decrease food intake, increase energy expenditure, and result in decreased adipose tissue mass. A critical aspect of this negative-feedback model is that hormones responsive to the level of adiposity inhibit anabolic pathways, while activating catabolic pathways. It is the balance between these two pathways that ultimately determines the animal's ingestive behavior and defended level of adiposity.

The catabolic and anabolic effector systems are in actuality a series of discrete neurotransmitter systems and axonal pathways in the brain. Many of the key details of this overall schema have emerged in the last few years. Although receptors for leptin and insulin are located throughout the CNS, both are concentrated in the ARC in the ventral hypothalamus. Hence, ARC neurons are

sensitive to these hormones and consequently to the amount of adipose tissue in the body.

A. ANABOLIC EFFECTOR SYSTEMS

The best-described anabolic effector peptide in the brain is NPY. Although NPY mRNA and peptide are distributed widely throughout the CNS, NPY-containing cell bodies in the ARC are especially important in the control of energy homeostasis (Schwartz *et al.*, 1992). Although these ARC NPYergic neurons directly influence several areas of the brain, major projections are to the nearby PVN and the lateral hypothalamic area (LHA). ARC NPYergic neurons respond to negative energy balance (e.g., food deprivation) by synthesizing more NPY mRNA and consequently release more NPY in the PVN (Kalra *et al.*, 1991; Schwartz *et al.*, 1992) and presumably the LHA as well. Importantly, animals in negative energy balance have low levels of adiposity hormones, resulting in elevated NPY mRNA in the ARC. Local replacement of either insulin or leptin in the vicinity of the ARC normalizes the elevated NPY mRNA in the ARC of fasted animals (Schwartz *et al.*, 1992; Sipols *et al.*, 1992). Hence, the activity of these ARC NPY neurons is under the direct influence of at least two adiposity signals.

Consistent with its being an anabolic effector peptide, administration of exogenous NPY into the PVN or into the adjacent i3vt elicits a rapid and robust increase in food intake (Clark *et al.*, 1984; Sahu and Kalra, 1993; Stanley *et al.*, 1993; Seeley *et al.*, 1995) and decrease in energy expenditure (Billington *et al.*, 1994). Repeated administration of NPY produces uncompensated increases in food intake, body weight, and adiposity. Local administration into the ARC of compounds that result in less NPY being synthesized result in reduced food intake and body weight (Akabayashi *et al.*, 1994). Hence, NPY meets all of the criteria of an anabolic effector peptide.

B. CATABOLIC EFFECTOR SYSTEMS

The catabolic counterpart of the ARC NPY system also appears to reside within the ARC. Considerable evidence implicates the ARC melanocortin system as an important catabolic effector system. Melanocortins are a family of peptides that include ACTH and α-MSH. The precursor molecule for ARC melanocortins is POMC. In addition to several other important neuropeptides, POMC encodes α-MSH, a transmitter that functions as an agonist at several classes of melanocortin receptors within the hypothalamus (especially within the PVN and LHA) (Cone, 1999,2000). When administered into the i3vt, α-MSH and other melanocortin receptor agonists (including the synthetic drug MTII) reduce food intake and body weight, whereas administration of synthetic melanocortin receptor antagonists (e.g., SHU-9119) increases food intake and body weight (Tsujii and

Bray, 1989; Fan *et al.*, 1997; Seeley *et al.*, 1997; Thiele *et al.*, 1998). POMC gene expression is reduced in negative energy balance (Schwartz *et al.*, 1997) and increased in positive energy balance (Hagan *et al.*, 1999).

Consistent with the hypothesis that the melanocortin system is important in mediating the effects of leptin, leptin receptors are found on POMC neurons, leptin stimulates POMC mRNA, and a melanocortin receptor antagonist blocks the effect of leptin to reduce food intake (Seeley *et al.*, 1997). Analogously, insulin receptors are found on POMC neurons, insulin stimulates POMC mRNA, and SHU 9119 attenuates insulin's anorexic action (Benoit *et al.*, 2002). All of this evidence points to the endogenous POMC/α-MSH/melanocortin receptor hypothalamic system as being a key catabolic effector pathway capable of eliciting robust effects on food intake and body weight that mediates the effect of adiposity signals in the CNS.

A compelling body of evidence implicates the hypothalamic melanocortin system as one of the central effectors controlling food intake and energy balance. The evidence for this is multifold. First, consider the phenotype of the agouti mouse. This yellow mouse has an autosomally transmitted trait resulting from a mutation of the agouti gene that results in ectopic expression of the agouti protein (Bultman *et al.*, 1992). In melanocytes, this inappropriate expression results in continuous antagonism of α-MSH signaling. The resulting phenotype is a yellow coat. However, the agouti mouse is obese as well as yellow. This observation led to the hypothesis that ectopic agouti protein antagonizes central melanocortin receptors involved in food intake. Indeed, though melanocortins have been known to influence food intake since the 1980s, it was only during the last decade that their receptors were cloned and localized to the hypothalamus. As predicted, agouti is an antagonist of these receptors (Lu *et al.*, 1994). Importantly, an endogenous AgRP subsequently was found to be produced almost exclusively in the ARC and to project to the PVN and LHA (Schwartz *et al.*, 1997).

Consistent with the hypothesis that the melanocortin system mediates the effects of adipose hormones is the finding that expression of melanocortin gene products is regulated by energy balance. During periods of negative energy balance (and, consequently, low adipose hormones), expression of AgRP mRNA is increased, while expression of POMC is decreased (Mizuno *et al.*, 1999). During positive energy balance (and high levels of adipose hormones), on the other hand, expression of POMC mRNA is increased and AgRP is decreased. Furthermore, as previously discussed, POMC-containing neurons also have receptors for leptin (Cheung *et al.*, 1997; Mountjoy and Wong, 1997; Seeley *et al.*, 1997). All these findings suggest that the hypothalamic melanocortin system is a likely central target of adipose signals and a mediator of their effects on food intake.

The brain expresses two types of melanocortin receptors, MC3R and MC4R (Cone, 1999,2000). Distribution of MC3R is limited to areas of the hypothala-

mus, while MC4R are located throughout the brain. Most data suggest that MC4R are most critical for melanocortin involvement in food intake. Indeed, an important piece of evidence linking melanocortins to food intake also supports the hypothesis that MC4R is the critical receptor. Mice with targeted deletion of the MC4R gene are phenotypically similar to the yellow (agouti) mouse (Huszar *et al.*, 1997). While not yellow, they exhibit profound obesity as well as hyperinsulinemia. As predicted, nonselective MC3/4R agonists (e.g., MTII) do not reduce food intake in MC4R-/- mice (Marsh *et al.*, 1999). While the issue is complicated by findings that MC3R-/- mice have increased body fat but not increased food intake (Chen and Marsh, 2000), such findings nonetheless support the hypothesis that the melanocortin system plays an important role in the control of energy balance.

Additional evidence supporting a role for melanocortins in the control of food intake comes from experimental administrations of both naturally occurring and synthetic peptides. i3vt administration of α-MSH decreases food intake (Tsujii and Bray, 1989; McMinn *et al.*, 2000), as does i3vt administration of synthetic agonists, including MTII and Ro27–3225 (Fan *et al.*, 1997; Thiele *et al.*, 1998; Benoit *et al.*, 2000). Conversely, administration of melanocortin receptor antagonists, such as AgRP or the synthetic agonist SHU-9119, elicits long-lasting increase in food intake (Fan *et al.*, 1997; Ollmann *et al.*, 1997; Rossi *et al.*, 1998; Hagan *et al.*, 1999,2000). While these data are consistent with the hypothesized role of the melanocortin system in the control of food intake, the presence of both an endogenous agonist and antagonist of the same receptor makes this system a prime candidate for the translation of negative-feedback signals from adipose tissue.

Figure 1 depicts a neuroanatomical schematic of the hypothalamic melanocortin system control of food intake. POMC neurons in the ARC express receptors for adiposity signals (e.g., leptin). These hormones convey an adiposity signal to the brain, which is in part received by ARC POMC neurons. To mediate the anorexic effects of leptin and insulin, these neurons, in turn, release α-MSH. Leptin and insulin also function to reduce NPY and AgRP expression. Figure 1 also depicts the most-characterized projection sites of these ARC POMC neurons, the PVN and the LHA, where the MC3/4R thought to be most important in the control of food intake and body weight are localized. However, relatively little experimental attention has been given to either 1) the downstream projections of these PVN neurons or 2) MC3/4R in other areas of the brain that might play important roles in the control of ingestion.

Importantly, the melanocortin system projects to multiple areas in the brain. Because food intake and body weight regulation are likely controlled by several CNS processes, the possibility remains that melanocortin peptides might alter food intake and body weight in multiple ways. For example, POMC-expressing neurons and the densest expression of MC4R actually are found in the caudal

brainstem (Kishi *et al.*, 2003). Additionally, MC4R are expressed in the infralimbic and insular cortices, LHA, bed nucleus of the stria terminalis, lateral parabrachial nucleus, nucleus of the solitary tract, hippocampus, amygdala, and the dorsal motor nucleus of the vagus (Lindblom *et al.*, 2002; Zhou *et al.*, 2002; Alvaro *et al.*, 2003; Kishi *et al.*, 2003). Thus, the melanocortin system may play important roles in the detection of interoceptive state signals (e.g., hunger and satiety), taste processing, or even reward or learning about ingested foods (Saper *et al.*, 2002).

VI. Synergy and Interactions of the Systems

Leptin and insulin fill distinct niches in the endocrine system. Although leptin has been implicated in several systemic processes (e.g., angiogenesis) (Schwartz *et al.*, 1996a), the primary role of leptin appears to be as a negative-feedback adiposity signal that acts in the brain to suppress food intake and net catabolic effector peptides (Porte *et al.*, 1998; Woods *et al.*, 1998,2003). Consistent with this, animals lacking leptin or functional leptin receptors are grossly obese. Insulin, in contrast, has a primary action in the periphery to regulate blood glucose and stimulate glucose uptake by most tissues. Analogous to leptin, however, deficits in insulin signaling are associated with hyperphagia in humans. Animals that lack normal insulin signaling in the brain are also obese.

The potential for redundancy between leptin and insulin has been highlighted by several recent studies in which leptin and insulin have been found to share intracellular and neuronal signaling pathways. While the melanocortin system has long been thought to mediate the central actions of leptin, recent studies in which insulin significantly stimulated POMC expression in fasted rats and insulin-induced hypophagia was blocked by a nonspecific melanocortin receptor antagonist (Fan *et al.*, 1997; Ollmann *et al.*, 1997; Seeley *et al.*, 1997; Rossi *et al.*, 1998; Hagan *et al.*, 1999,2000) strongly support a role for the melanocortin system in the regulation of energy balance by insulin as well. Furthermore, phosphatidylinositol-3-OH kinase (PI3-K), an enzyme that is an intracellular mediator of insulin signaling, appears to play a crucial role in the leptin-induced anorexia signal transduction pathway (Niswender and Schwartz, 2003). While these data are consistent with the concept that leptin and insulin share such pathways, they also suggest that, over time, this redundancy dissipates and their pathways diverge.

VII. Additional Complexity of the Leptin/Insulin-Melanocortin System

Syndecans are a family of highly abundant cell-surface heparan sulfate proteoglycans (HSPGs) (proteins with covalently attached, highly acidic sugar chains) that are unique in their ability to bind extracellular peptides such as

hormones and growth factors. They act as coreceptors by modulating interactions of peptide ligands with their activity-generating receptors. In mammals, the syndecans are comprised of four transmembrane HSPGs and members of the glypican family of glycerophosphoinositol-linked HSPGs. Together, they account for nearly all HSPGs unbiquitously expressed at cell surfaces (Bernfield *et al.*, 1999; Park *et al.*, 2000). Syndecan family members are found on virtually every cell type (Bernfield *et al.*, 1999) but are differentially expressed in a tissue-specific manner. Syndecan-1 is found predominately on epithelial cells, while syndecan-3 is found primarily on neural crest-derived cells and neurons. They are induced during development and injury and in response to a wide spectrum of physiological stimuli (Lauri *et al.*, 1998; Bernfield *et al.*, 1999; Hsueh and Sheng, 1999).

During the course of studies on the function of syndecan-1, Reizes and colleagues (2001) discovered that the syndecans play an important role in the regulation of food intake and body weight. In those studies, they induced overexpression of syndecan-1 in mice. For reasons that still are not clear, this did not result in ubiquitous expression but rather yielded high levels of syndecan-1 expression in specific areas of the brain where it would normally not be found. In particular, the syndecan-1 transgene was highly expressed in hypothalamus. Syndecan-1 transgenic mice have severe maturity-onset obesity and type II diabetes. The phenotype of this obesity closely resembles that of previously characterized mice with disruptions of the melanocortin signaling pathway, including the agouti yellow, AgRP overexpressers, and MC4R knockout mice (Huszar *et al.*, 1997; Ollmann *et al.*, 1997). Additionally, syndecan-1 was found to potentiate the obesity of the yellow (Ay/a) mice and to potentiate the activity of AgRP and agouti signaling protein in cell-culture preparations. Finally, it was discovered that syndecan-1 promoted obesity only when it was expressed at the cell surface. Mice that constitutively shed syndecan-1 because the membrane-binding region of the gene had been deleted had a complete reversal of the obese phenotype (Reizes *et al.*, 2001). These findings are consistent with the hypothesis that the syndecan-1 transgene acts as a coreceptor for AgRP on MC3R- and MC4R-containing neurons. This, in turn, led to the hypothesis that syndecan-3, normally expressed in hypothalamic tissues, is a coreceptor for endogenous AgRP antagonism.

VIII. Dysregulation of Energy Homeostasis

Obesity is increasing and has been declared an epidemic. The epidemiological data are quite compelling and have been summarized in several reviews (Bray and Popkin, 1998; Samaras *et al.*, 1998). Additionally, studies in animals provide strong experimental evidence that increasing dietary fat accelerates the development of obesity. Across numerous experiments, diets, and species, the

conclusion that increased consumption of high-fat (HF) diets leads to increased body fat is inescapable. There are many reviews of this literature (Hill *et al.*, 1992; Warwick and Schiffman, 1992; Warwick, 1996; Golay and Bobbioni, 1997; West and York, 1998) and several valuable points have emerged from them. Perhaps most importantly, strong genetic influences dictate whether or not a given individual will be prone or resistant to becoming obese when exposed to a HF diet (Levin and Routh, 1996; West, 1996; Leibel *et al.*, 1997; Levin *et al.*, 1997; Reed *et al.*, 1997; West and York, 1998). As Bray and Popkin point out, a HF diet can be viewed as the environmental agent that acts on a susceptible host animal to produce the noninfectious disease, obesity (Bray *et al.*, 1990).

Experiments in which animals were rendered obese, then placed on a low(er)-fat diet, have been somewhat equivocal, with some reporting loss of body weight (Hill *et al.*, 1992) and others no weight reduction (Faust *et al.*, 1978; Rolls *et al.*, 1980; Harris *et al.*, 1986; Uhley and Jen, 1989; Hill *et al.*, 1992). One important parameter is evidently the age at which the obesity is initially induced. Younger rats (as well as older rats made obese by maintenance on a HF diet for longer intervals) may increase their number of adipocytes (Lemonnier, 1972; Faust *et al.*, 1978; Hill *et al.*, 1992). When subsequently placed on a low-fat (LF) diet, such animals tend not to lose weight (or body fat). However, if the number of adipocytes does not increase, obese animals placed on a LF diet lose weight to the level of rats never made obese at all (Hill *et al.*, 1992). Without assessing fat cell number, we have observed that adult rats given a HF diet and held at an obese weight for a prolonged interval lose weight to control levels when returned to a LF diet. One conclusion that has been reached from this literature is that it is easier to induce obesity in a lean individual with a HF diet than it is to induce leanness in an obese individual with a LF diet (Hill *et al.*, 1992; Bray and Popkin, 1998).

Importantly, the consequences of obesity, including dietary-induced obesity, are well documented and include type II diabetes and insulin insensitivity. Furthermore, some detrimental effects of dietary fat are not limited to obese individuals. For example, we recently demonstrated that while dietary-induced obesity decreases central sensitivity to the anorexic effects of central insulin administration, increased dietary fat, in the absence of frank obesity, attenuates the potency of central insulin to reduce food intake and body weight.

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