

Leptin and the Cardiovascular System

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ABSTRACT

Obesity is associated with increased cardiovascular morbidity and mortality, in part through development of hypertension. Recent observations suggest that the cardiovascular actions of leptin may help explain the link between excess fat mass and cardiovascular diseases. Leptin is an adipocyte-derived hormone that acts in the central nervous system to promote weight loss by decreasing food intake and increasing metabolic rate. Leptin causes a significant increase in overall sympathetic nervous activity, which appears to be due to direct hypothalamic effects and is mediated by neuropeptide systems such as the melanocortin system and corticotropin-releasing hormone. Renal sympathoactivation to leptin is preserved in the presence of obesity, despite resistance to the metabolic effects of leptin. Such selective leptin resistance, in the context of circulating hyperleptinemia, could predispose to obesity-related hypertension. Some *in vitro* studies have suggested that leptin may have peripheral actions such as endothelium-mediated vasodilation that might oppose sympathetically induced vasoconstriction. However, we and others have shown that leptin does not have direct vasodilator effects *in vivo*. The fact that chronic leptin administration or overexpression of leptin produces hypertension supports the concept that the hemodynamic actions of leptin are due predominantly to sympathetic activation. Exploration of the sites and mechanisms of leptin resistance should provide novel therapeutic strategies for obesity, insulin resistance, and hypertension.

I. Introduction

Obesity has become one of the most-serious health problems in industrialized societies. Weight gain is associated with a high risk of developing cardiovascular and metabolic diseases such as coronary heart disease, hypertension, diabetes, and dyslipidemia. Epidemiological studies have documented a close relationship between body mass index (BMI) and cardiovascular events (Hubert *et al.*, 1983; Kushner, 1993; Kopelman, 2000). The association between body weight and blood pressure has been found even in normotensive subjects with normal BMI (Stamler *et al.*, 1978; Mikhail and Tuck, 2000). Subsequently, clinical studies have demonstrated that weight loss induced by low-calorie diet or gastric bypass reduces arterial pressure and corrects diabetes and other comorbidities associated with obesity (Carson *et al.*, 1994; Richards *et al.*, 1996). Several experimental models of obesity-induced hypertension have been devel-

oped. Different species — including the dog, rabbit, rat, and mouse — develop obesity associated with an increase in blood pressure when fed a high-fat diet (Kaufman *et al.*, 1991; Mills *et al.*, 1993; Montani *et al.*, 2002). Some genetic models of obesity (e.g., the Zucker fatty rat, agouti obese mouse) are also used as models of obesity hypertension (Kurtz *et al.*, 1989; Mark *et al.*, 1999).

Until recently adipose tissue was considered exclusively as a body energy store without other function. However, the realization that adipocytes can produce many hormones — including leptin, adiponectin, resistin, atrial natriuretic peptide, and angiotensinogen — have led to a view that this tissue is an endocrine secretory organ (Bradley *et al.*, 2001). Hormones produced by the adipose tissue may act locally to affect adipocyte growth and differentiation or may be released into the circulation to act elsewhere. Among the different hormones released by adipose tissue, leptin has probably drawn the most attention in recent years.

II. Leptin

Classical lesion studies established the importance of the hypothalamus in control of energy homeostasis (for a review, see Elmquist *et al.*, 1999). It was then postulated that to control body fat stores, the brain must receive afferent input in proportion to the current level of body fat. Coleman (1973) was the first to demonstrate the existence of a circulating factor that plays a major role in the regulation of body weight. Using parabiosis methodology, he found that obesity in the ob/ob mouse was due to lack of this factor, whereas obesity in the db/db mouse was caused by reduced sensitivity to the factor. The much-later cloning of the ob gene revealed the identity of the adiposity factor, which was termed “leptin” (Zhang *et al.*, 1994). This hormone is a 167-amino acid protein expressed mainly by adipocytes and released in the blood in proportion to the size of adipose tissue, which is consistent with leptin’s role as a signal of the energy stores (Figure 1). Leptin gene expression and secretion are increased by overfeeding, high-fat diet, insulin, glucocorticoids, endotoxin, and cytokines and decreased by fasting, testosterone, thyroid hormone, and exposure to cold (Coleman and Hermann, 1999; Fried *et al.*, 2000). Leptin gene expression also is modulated by the sympathetic nervous system. For example, we found that blockade of norepinephrine synthesis with alpha-methyl-para-tyrosine or pharmacologic sympathectomy with 6-hydroxydopamine increases leptin mRNA as well as plasma leptin (Sivitz *et al.*, 1999). These observations suggest that the sympathetic nervous system tonically inhibits leptin gene expression through a negative-feedback loop to adipose tissue.

In lean subjects, leptin circulates at low levels (i.e., 5–15 ng/ml) as a free form and attached to binding proteins (Sinha *et al.*, 1996). Plasma leptin is transported to the central nervous system (CNS) by a saturable, unidirectional

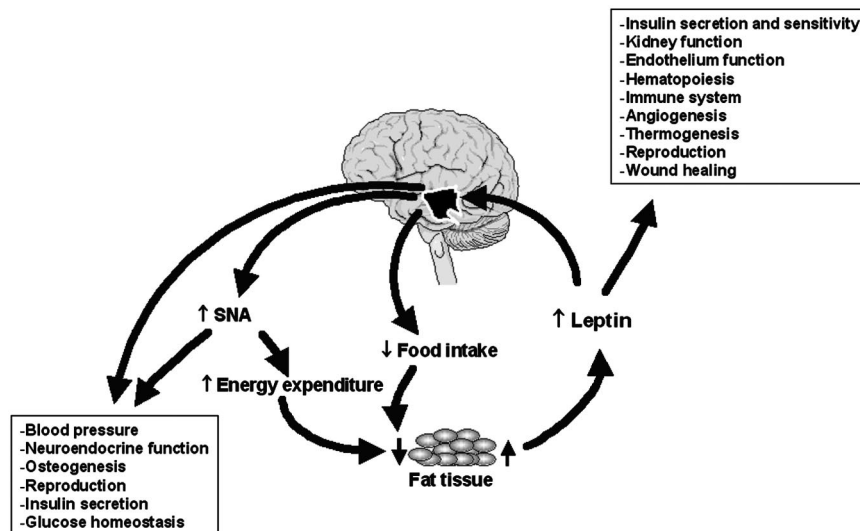


FIG. 1. Role of leptin in the regulation of body weight and other functions. Leptin is secreted by adipocytes and circulates in the blood in a concentration proportional to fat mass content. Action of leptin on its receptor present in the hypothalamus inhibits food intake and increases energy expenditure through stimulation of sympathetic nerve activity (SNA). Leptin modulates different other functions by direct peripheral action in various tissues or through the modulation of SNA.

system (Banks *et al.*, 1996), involving binding of leptin to the short form of the leptin receptor located at the endothelium of the vasculature and the epithelium of choroid plexus (Bjorbaek *et al.*, 1998a). Some evidence suggests that leptin also may be produced in the brain (Wiesner *et al.*, 1999); however, the physiological role of leptin produced locally in the brain and its autocrine and/or paracrine effects remain unknown. Leptin action in the CNS promotes weight loss by decreasing food intake and increasing energy expenditure (Figure 1). The severe obesity and hyperphagia caused by the absence of leptin in rodents and humans demonstrate the importance of this hormone for the control of body weight and food intake (Friedman and Halaas, 1998). Although the main role of leptin is as an adipostat, more-recent studies have shown that leptin has a broad range of effects in different tissues (Wauters *et al.*, 2000; Margetic *et al.*, 2002). Leptin affects these different functions either by direct action in peripheral tissues or through its action in the CNS (Figure 1).

III. Leptin Receptor and Mechanisms of Signaling

The leptin receptor is a protein with a single transmembrane domain belonging to the cytokine superfamily. Six different alternatively spliced iso-

forms of this receptor have been identified (designated Ob-Ra to Ob-Rf) (Tartaglia, 1997). Five isoforms (Ob-Ra to Ob-Rd and Ob-Rf) share an identical extracellular domain but differ in the length of their intracellular domain. Ob-Re, which lacks both the transmembrane and intracellular domains, is a soluble form of the receptor. The Ob-Rb form that encodes the full receptor, including the long intracellular domain, appears to mediate most of the biological effects of leptin (Chen *et al.*, 1996; Tartaglia, 1997). As predicted by parabiosis studies, the db/db mouse lacks the Ob-Rb form of the receptor, resulting from premature stop codon at the 3'-end of the Ob-Rb transcript (Chen *et al.*, 1996; Chua *et al.*, 1996). In contrast, the obese Zucker rat lacks all forms of the leptin receptor, caused by an amino acid substitution (glutamine \rightarrow proline) in the extracellular domain common to all known isoforms of the leptin receptor (Iida *et al.*, 1996; Phillips *et al.*, 1996).

The janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway was the first signaling mechanism associated with the leptin receptor (Vaisse *et al.*, 1996). Activation of this signaling pathway in the hypothalamus is initiated upon the conformational changes in the leptin receptor triggered by leptin binding. Intracellular JAK proteins (JAK2 and possibly JAK1) that are associated with the binding motifs located in the proximal region of the leptin receptor then are activated by transphosphorylation. Activated JAK proteins, in turn, phosphorylate tyrosine residues of the receptor, providing docking sites for STAT proteins, which become tyrosine-phosphorylated by JAK. Phosphorylated STAT molecules dimerize and translocate to the nucleus to modulate the transcription of target genes (Figure 2). While *in vitro* studies have shown that leptin receptors signal through different STAT molecules, *in vivo* studies have demonstrated that, in the hypothalamus, this occurs specifically through activation of STAT3 (Bates *et al.*, 2003). Leptin's effects on the newly identified suppressors of the cytokine signaling family (SOCS) represent a negative-feedback mechanism for this pathway (Bjorbaek *et al.*, 1998b; Emilsson *et al.*, 1999). SOCS proteins negatively regulate the JAK/STAT pathway, either by directly blocking JAKs or through inhibition of further STAT phosphorylation.

More recently, several other intracellular signaling mechanisms have been associated with the leptin receptor. By engaging JAK2, the leptin receptor is able to stimulate insulin receptor substrate (IRS)-2, which, in turn, activates phosphoinositol-3 kinase (PI₃-K) through an association to its regulatory subunit (Niswender *et al.*, 2001). This pathway appears to be involved not only in the modulation of neuronal firing rate via the activation of the potassium-adenosine triphosphate (ATP) channels (Niswender and Schwartz, 2003) but also in the modulation of gene transcription through a phosphodiesterase 3-cyclic adenosine monophosphate (cAMP) mechanism (Zhao *et al.*, 2002). The leptin receptor also was found to be able to activate the extracellular factor-regulated kinases

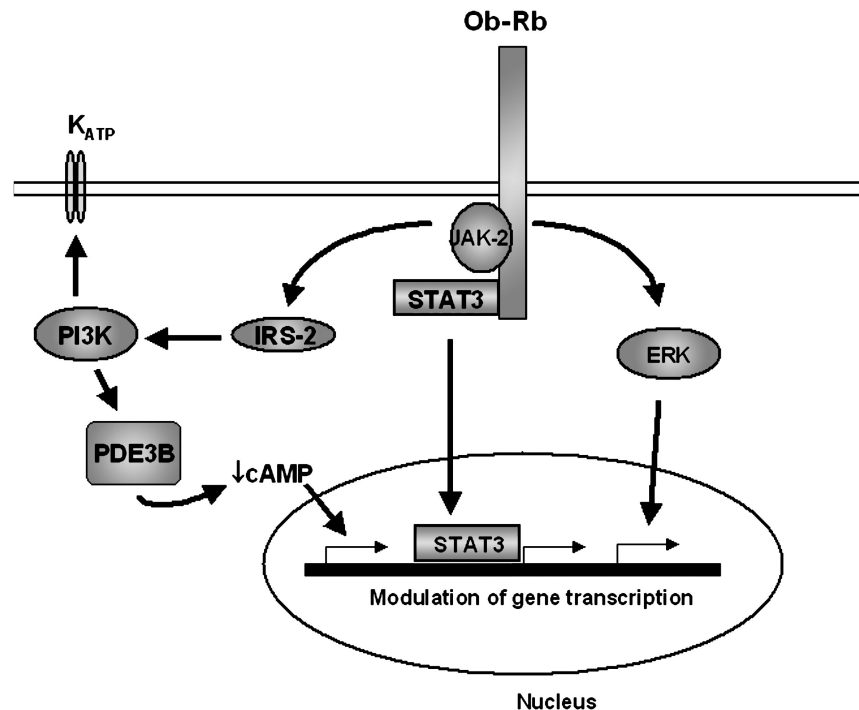


FIG. 2. Molecular mechanisms involved in leptin receptor (Ob-Rb) signaling in the hypothalamus. Leptin modulates gene transcription via activation of signal transducer and activator of transcription (STAT) proteins, phosphoinositol 3 kinase (PI3-K, via the phosphodiesterase 3B (PDE3B)), and a member of the mitogen-activated protein kinase, extracellular factor-regulated kinase (ERK). The PI3-K pathway also appears to be involved in the modulation of neuronal firing rate via the activation of the potassium-adenosine triphosphate channels (K_{ATP}).

(ERKs), a member of the mitogen-activated protein kinase (MAPK) (Bjorbaek *et al.*, 2001; Bates *et al.*, 2003), by different mechanisms, including direct phosphorylation by JAK2. This ERK pathway appears to be involved in leptin-induced immediate early-response gene expression, *c-fos*.

IV. Leptin and the Sympathetic Nervous System

A. SYMPATHETIC EFFECTS OF LEPTIN

Consistent with its role in the regulation of energy expenditure, leptin was found to increase norepinephrine turnover in brown adipose tissue (BAT) (Collins *et al.*, 1996), suggesting activation of sympathetic outflow to this tissue.

Using multifiber recording of regional sympathetic nerve activity (SNA), we evaluated the effects of leptin on the sympathetic outflow to different beds (Haynes *et al.*, 1997). As expected, we found that intravenous administration of leptin in anesthetized Sprague-Dawley rats caused a significant and dose-dependent increase in SNA to BAT (Figure 3). Unexpectedly, leptin caused sympathoactivation to other beds not usually considered thermogenic, such as the kidney, hindlimb, and adrenal. Satoh *et al.* (1999) investigated the effect of leptin on circulating catecholamines and found that leptin administration caused a significant and dose-dependent increase in plasma concentration of norepinephrine and epinephrine.

We have shown that the leptin-induced regional increases in SNA respond nonuniformly to baroreflex activation and hypothermia (Hausberg *et al.*, 2002a,b). Leptin-induced increases in renal SNA can be suppressed by baroreflex activation, suggesting that the increase in renal SNA subserves circulatory functions. In contrast, leptin-induced BAT sympathoactivation was not prevented by baroreflex activation, suggesting the recruitment of sympathetic fibers that serve thermogenic or metabolic, rather than circulatory, functions (Hausberg *et al.*, 2002a). The effect of leptin on regional SNA response to hypothermia differs

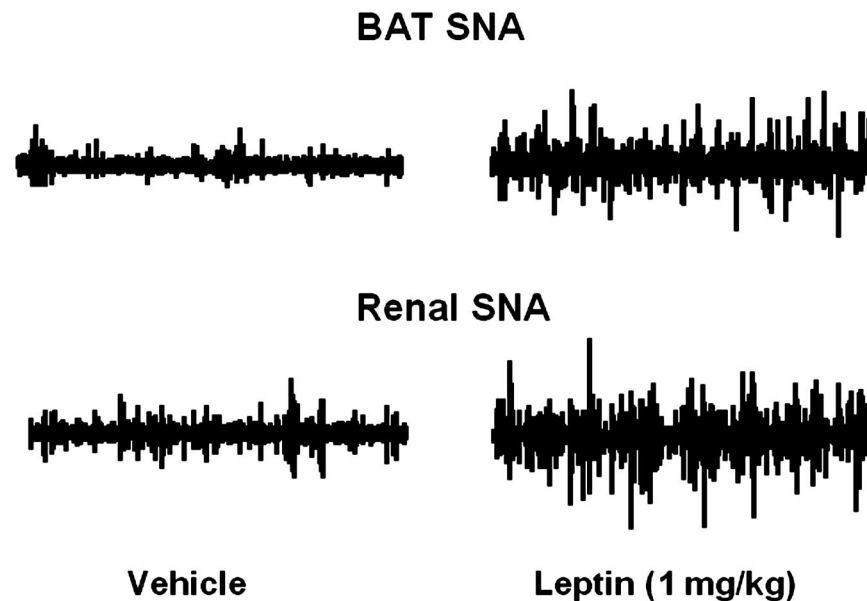


FIG. 3. Effects of intravenous administration of leptin (1 mg/kg), as compared to vehicle, on SNA to brown adipose tissue (BAT) and kidney in Sprague-Dawley rats. Leptin caused significant increases in both renal and BAT SNA.

between sympathetic fibers that serve circulatory or thermogenic functions. Leptin, at a low dose that does not alter baseline SNA, acutely enhances sympathetic outflow to BAT in response to hypothermia in lean rats. This effect is specific for thermogenic SNA because leptin does not affect the response of renal SNA to hypothermia (Hausberg *et al.*, 2002b).

Although some reports have shown that leptin could increase SNA through stimulation of peripheral afferent nerves (Nijima *et al.*, 1998; Tanida *et al.*, 2000), our data support the concept that sympathoactivation to leptin is due to the action of this hormone in the CNS. First, leptin-induced sympathoexcitation remains apparent after transection of the sympathetic nerves distal to the recording site and disappears after ganglion blockade with intravenous chlorisondamide (Haynes *et al.*, 1997). These findings indicate that the increase in SNA resulted from efferent sympathetic nerves rather than afferent nerves. Second, direct administration of leptin to the third cerebral ventricle, at sub-systemic doses, increases SNA (Haynes, 2000) and dose-dependently increases plasma catecholamines (Sato *et al.*, 1999). Third, sympathoactivation to intravenous leptin can be abolished completely by selective lesioning of the hypothalamic arcuate nucleus (Haynes, 2000). The arcuate nucleus of the hypothalamus is also considered the major site of leptin action to control body weight and food intake (Schwartz *et al.*, 2000).

In humans, there is no direct evidence for a role of leptin in the regulation of the sympathetic nervous system because results from leptin infusions in humans are lacking. In nonhuman primates, however, leptin has been shown to activate the sympathetic nervous system, as assessed by an increase in circulating norepinephrine levels after a single cerebroventricular administration of leptin (Tang-Christensen *et al.*, 1999). Indirect evidence suggests that leptin may be important for control of SNA in humans. A positive and significant correlation between muscle SNA and plasma leptin concentration has been reported in healthy, nondiabetic men (Snitker *et al.*, 1997). Also, serum leptin levels are a strong positive determinant of resting metabolic rate, which is under sympathetic control, suggesting that action of leptin on SNA is a determinant of energy expenditure in humans. In addition, Jeon *et al.* (2003) have shown that the correlation between leptin and resting metabolic rate is lost in patients with a disrupted sympathetic nervous system caused by spinal cord injury. These patients had also a lower resting metabolic rate. Together, these findings strongly support the concept that leptin influences energy expenditure through the sympathetic nervous system in humans.

B. SYMPATHETIC EFFECTS OF LEPTIN IN OBESITY

Several lines of evidence suggest that obesity is associated with activation of the sympathetic nervous system. Plasma and urinary catecholamines are in-

creased in obese humans as well as in obese animal models (Sowers *et al.*, 1982; Young and Landsberg, 1982; Rocchini *et al.*, 1989). Using direct measurement with microneurography, several groups have shown increased SNA to skeletal muscle in obese subjects, as compared to lean individuals (Vollenweider *et al.*, 1994; Grassi *et al.*, 1995). Norepinephrine spillover techniques have demonstrated that human obesity is associated with increased SNA to a key organ of the cardiovascular homeostasis, the kidney (Vaz *et al.*, 1997). Elevated renal SNA is also reported in animal models of obesity, including rats on high-fat diet (Iwashita *et al.*, 2002). These findings demonstrate that enhanced SNA is a common feature of obesity, which would play a major role in obesity-induced hypertension and cardiovascular disease (Hall *et al.*, 2000). However, the mechanisms responsible for increased SNA in obesity remain unknown.

Obesity is known to be associated with circulating hyperleptinemia, reflecting resistance to leptin because, despite high circulating levels of leptin, such subjects remain obese (Considine *et al.*, 1996). Under these circumstances, in order for leptin to have a role in obesity-related hypertension, one must postulate that any leptin resistance is selective, with preservation of sympathetic responsiveness. Indeed, we have demonstrated that in some animal models, including agouti mice and diet-induced obesity, leptin resistance is selective, with sparing of the effects of leptin on renal SNA. For example, in agouti mice, the anorexic and weight-reducing effects of leptin were less in the obese mice, compared to lean littermates. However, the increase in renal SNA in response to leptin was identical in both lean and obese mice (Correia *et al.*, 2002; Rahmouni *et al.*, 2002). Eikelis *et al.* (2003) recently have shown the existence of a strong correlation between leptin plasma concentration and renal SNA across a broad range of leptin values in men of widely differing adiposity. This indicates that leptin may be the main cause of sympathoactivation associated with obesity in both animal models and humans (Figure 4).

C. MECHANISMS OF LEPTIN-INDUCED SYMPATHOACTIVATION

The receptor-mediated sympathoexcitatory effect of leptin is supported by the absence of SNA response to leptin in obese Zucker rats (Haynes *et al.*, 1997). However, it was not clear which form of the leptin receptor was involved, since Zucker rats lack all forms of the leptin receptor. We recently demonstrated an absence of renal SNA response to leptin in db/db mice that indicates that the effects of leptin on sympathetic outflow are mediated by the long-form Ob-Rb of the leptin receptor (Rahmouni *et al.*, 2003b).

As mentioned earlier, the leptin receptor has divergent signaling capacities and modulates the activity of different intracellular enzymes. Although STAT signaling was thought to be the main pathway that mediates the leptin action in the hypothalamus, PI₃-K has been found to play a pivotal role in the effect of

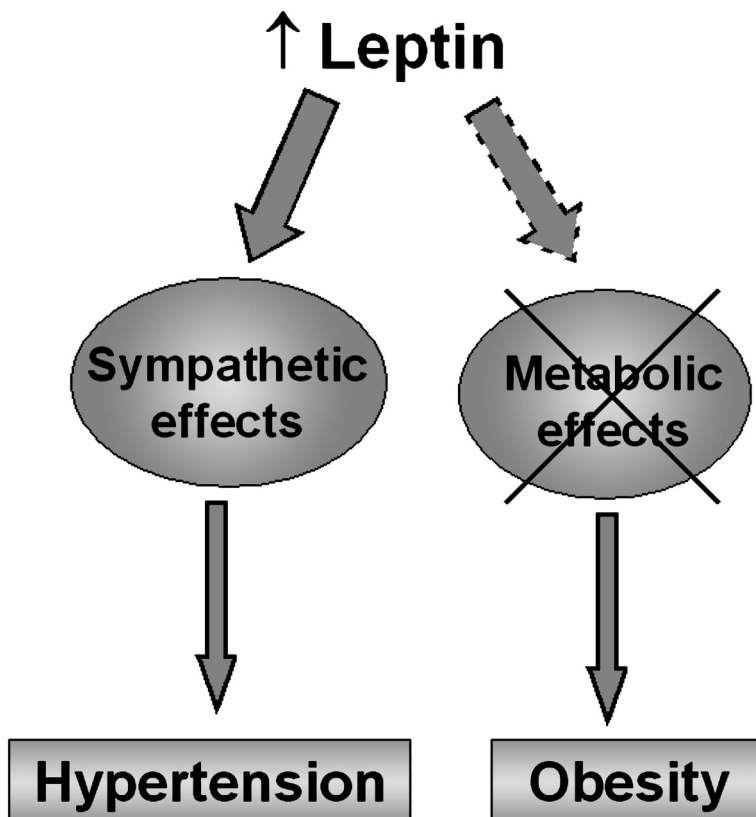


FIG. 4. Concept of selective leptin resistance: there is resistance to the appetite- and weight-reducing actions of leptin but preservation of the sympathetic actions. This phenomenon might explain in part how hyperleptinemia could be accompanied by obesity (partial loss of appetite and metabolic actions of leptin) but still contribute to sympathetic overactivity and hypertension because of preservation of the sympathetic actions of leptin to some organs involved in blood pressure regulation (e.g., kidney).

leptin on food intake (Niswender and Schwartz, 2003). Our group has demonstrated that PI_3 -K plays a major role in the transduction of leptin-induced changes in renal sympathetic outflow. We compared renal sympathoactivation to leptin before and after intracerebral administration of PI_3 -K inhibitors. Both LY294002 and wortmannin markedly attenuated the increase in renal SNA induced by leptin, without affecting sympathoactivation to stimulation of the melanocortin system (Rahmouni *et al.*, 2003a). The role of PI_3 -K and other pathways in leptin-induced sympathoactivation to different other beds — including BAT, hindlimb, and adrenal gland — remains unknown. Leptin likely controls sym-

pathetic nerve activity in a tissue-specific manner, for several reasons. First, activation of arterial baroreceptors and hypothermia modulate differentially leptin-induced sympathoactivation to the kidney and BAT (Hausberg *et al.*, 2002a,b). Second, in diet-induced obese mice, lumbar SNA responses to leptin are attenuated, as compared to lean mice, whereas leptin-induced increases in renal SNA occur with the same time course and magnitude in both diet-induced obese and lean mice (K. Rahmouni, D.A. Morgan, A.L. Mark, W.G. Haynes, unpublished data).

Several hypothalamic neuropeptides, monoamines, and other transmitter substances have been identified as candidate mediators of leptin action in the hypothalamus. These include melanocortins, neuropeptide Y (NPY), corticotropin-releasing hormone (CRH), melanin-concentrating hormone, and cocaine- and amphetamine-regulated transcript (CART) (Schwartz *et al.*, 2000). Therefore, leptin could cause regional sympathoactivation through stimulation of different neuropeptides. In the neural melanocortin system, alpha-melanocyte stimulating hormone (α -MSH) is derived from pro-opiomelanocortin (POMC) and acts mainly on melanocortin-4 receptors (MC-4R). Both renal and lumbar sympathoactivation to leptin seem mediated by the melanocortin system because blockade of melanocortin receptors with SH9119 (Haynes *et al.*, 1999) or agouti protein (Dunbar and Lu, 1999) inhibits the renal and lumbar SNA response to leptin. However, SHU9119 does not block leptin-induced sympathoactivation to BAT (Haynes *et al.*, 1999). Using a MC-4R knockout mouse, we recently confirmed that the renal SNA response to leptin is mediated by MC-4R. Indeed, we have shown a gene dose effect, with MC4-R heterozygotes having 50% of the normal response to leptin and homozygote knockouts having no renal SNA response to leptin (Rahmouni *et al.*, 2003b). The interrelationship between leptin and the melanocortin system appears to be more complex than first thought because absence of the leptin receptor in db/db mice attenuates the renal SNA response to stimulation of the MC-3/4R with MTII (Rahmouni *et al.*, 2003b). This was not expected because the melanocortin system was considered downstream to leptin. Although the mechanisms of this attenuated SNA response to MTII in db/db mice remain unknown, one possibility relates to the increased expression of the agouti-related protein in these mice (Korner *et al.*, 2001), which is known to at least partially block the melanocortin receptors in the brain.

The increase in BAT SNA seems to depend on neuropeptides other than the melanocortin system. Given that intracerebral CRH increases SNA to BAT, we investigated the role of this system in leptin-induced sympathoactivation to this tissue. Our results show that a CRH receptor antagonist blocked leptin-induced sympathoactivation to BAT but not to the kidney (Correia *et al.*, 2001a). In summary, leptin appears to cause regional sympathoactivation via different neuropeptide pathways, with melanocortins mediating renal sympathoactivation and CRF mediating BAT SNA to leptin.

V. Leptin and Blood Pressure

A. PRESSOR EFFECTS OF LEPTIN

Leptin-induced activation of SNA to organs such as the kidney was the first indication of the potential role of this hormone in regulation of blood pressure. The sympathetic nervous system is an important component in the control of renal function (DiBona and Kopp, 1997). Long-term renal sympathetic stimulation by leptin would be expected to raise arterial pressure by causing vasoconstriction and by increasing renal tubular sodium reabsorption. Dunbar *et al.* (1997) have shown that the sympathoactivation to leptin is followed by a slow but progressive increase in mean arterial pressure. Shek *et al.* (1998) demonstrated that intravenous infusion of leptin at a dose that increased plasma leptin from 1 to 94 ng/ml for 12 days increased arterial pressure and heart rate, despite a decrease in food intake that would be expected to decrease arterial pressure. Leptin-induced increases in arterial pressure probably are due to a central neural action of this hormone because intracerebroventricular administration of leptin mimics the effects of systemic administration (Correia *et al.*, 2001b). The substantial dose-dependent increase in heart rate, as well as the greater response to air-jet stress observed in leptin-treated rats, supports central activation of the sympathetic nervous system (Correia *et al.*, 2001b). Finally, blockade of the adrenergic system inhibits the pressor response to leptin (Carlyle *et al.*, 2002).

Further evidence for the pressor effects of leptin derives from studies of transgenic mice overexpressing leptin in the liver (Aizawa-Abe *et al.*, 2000). These mice had 10-fold increases in plasma leptin and decreased body weight. Despite the decreased body weight, the transgenic mice overexpressing leptin had significantly higher arterial pressure than nontransgenic littermates. The transgenic mice also had increased urinary excretion of norepinephrine, a marker of sympathetic nervous system activity. The increase in arterial pressure was normalized after alpha-adrenergic or ganglionic blockade, again demonstrating the importance of the sympathetic nervous system in the pressor effects of leptin.

We evaluated arterial pressure in obese, leptin-deficient *ob/ob* mice and their wild-type, lean controls (Mark *et al.*, 1999). Despite body weights nearly twice as high as their lean controls, the leptin-deficient *ob/ob* mice had lower arterial pressure. Aizawa-Abe *et al.* (2002) subsequently reported that administration of leptin to *ob/ob* mice (so-called leptin reconstitution) increased systolic blood pressure by as much as 25 mmHg, despite decreases in food intake and body weight. These findings demonstrate that leptin contributes physiologically to the regulation of arterial pressure.

In contrast to leptin-deficient *ob/ob* mice, agouti yellow obese mice have elevated arterial pressure, despite the fact that they have milder obesity than *ob/ob* mice (Mark *et al.*, 1999). Obesity induced by a high-fat diet is also

associated with an increase in arterial pressure (Mills *et al.*, 1993; Iwashita *et al.*, 2002). The presence of high circulating levels of leptin associated with the selectivity in leptin resistance (i.e., preserved ability of leptin to increase renal SNA) (Figure 4) could explain the hypertension in these different models of obesity. Other mechanisms may contribute to the development of obesity-related hypertension. For example, *in vitro* studies have shown that leptin causes oxidative stress in cultured endothelial cells by increasing the generation of reactive oxygen species (ROS) (Bouloumie *et al.*, 1999; Yamagishi *et al.*, 2001). Leptin also has been shown to stimulate the secretion of proinflammatory cytokines (e.g., tumor necrosis factor- α , interleukin-6) that are known to promote hypertension (Loffreda *et al.*, 1998).

As has been described for renal SNA, the melanocortin system appears to mediate the effect of leptin on blood pressure. First, pharmacological activation of melanocortin receptors for 14 days caused significant increases in arterial pressure, despite decreases in food intake and body weight (Kuo *et al.*, 2003). Second, inhibition of melanocortin receptors blocks the increase in blood pressure induced by leptin (Dunbar and Lu, 1999).

In order to investigate whether the increase in arterial pressure induced by leptin is due to enhanced salt sensitivity, we studied the effects of a high-salt diet on the pressor responses of intracerebroventricular administration of leptin. The increase in arterial pressure was similar in leptin-treated rats fed a low- or high-salt diet, indicating that leptin-dependent mechanisms in the CNS do not alter arterial pressure sensitivity to salt (Correia *et al.*, 2001b).

In humans, several studies have shown that plasma leptin is related to blood pressure in both normotensive and hypertensive subjects (Hirose *et al.*, 1998; Schorr *et al.*, 1998). A positive correlation has been observed between longitudinal changes in leptin and the arterial pressure (Itoh *et al.*, 2002). Farooqi *et al.* (1999) have reported that replacement leptin therapy in a child with congenital leptin deficiency for 1 year caused a drastic decrease in body weight (16 kg). This weight loss would be expected to lower arterial pressure substantially but the arterial pressure did not change. These observations are consistent with a pressor action of leptin offsetting the depressor action of weight loss.

B. DEPRESSOR EFFECTS OF LEPTIN

Recently, several studies have suggested that leptin may have direct vascular effects that tend to decrease arterial pressure. The vascular endothelium is an important component in the control of arterial pressure homeostasis (Contreras *et al.*, 2000). Endothelial cells release several vasoactive factors, of which nitric oxide (NO) is perhaps the most important with potent vasodilator action. Functionally competent leptin receptors are present on endothelial cells (Sierra-Honigmann *et al.*, 1998) and leptin administration in rat causes a dose-dependent

increase in NO metabolite concentrations. In one study in anesthetized rats, infusion of leptin during inhibition of NO synthesis increased arterial pressure (Fruhbeck, 1999). Leptin also decreased arterial pressure after suppression of sympathetic influence using ganglionic blockade (Fruhbeck, 1999) or chemical sympathectomy (Lembo *et al.*, 2000). Furthermore, *in vitro* studies have shown that leptin evokes an endothelium-dependent relaxation of arterial rings (Kimura *et al.*, 2000; Lembo *et al.*, 2000). Therefore, it has been argued that these vasodilator effects of leptin might oppose its neurogenic pressor action.

In contrast to these reports, Gardiner *et al.* (1999) found no evidence for a vasodilator action of leptin in conscious rats. These authors showed that leptin does not change blood flow in different beds, including renal, mesenteric, and abdominal arteries. The presence of the NO synthase inhibitor, L-NAME, failed to unmask any pressor effect of leptin (Gardiner *et al.*, 1999). Similarly, we found that leptin does not have substantial direct or indirect vasodilator effects *in vivo*. Indeed, leptin, at a concentration sufficient to increase sympathetic nerve outflow, did not change arterial pressure or blood flow measured from the mesenteric, lower aortic, and renal arteries (Mitchell *et al.*, 2001). Blockade of the adrenergic system or NO synthase did not reveal any pressor effect of leptin (Mitchell *et al.*, 2001). Furthermore, leptin did not alter the sympathetically mediated vasomotor response in hindlimb or kidney to stimulation of the splanchnic sympathetic nerve trunk (Jalali *et al.*, 2001). Kuo *et al.* (2001) found that blockade of NO synthesis augmented the heart rate and renal vascular and glomerular responses to leptin but did not substantially augment the pressor response to leptin. Thus, the role of NO in blood pressure responses to leptin remains controversial but consistent negative results of studies in conscious animals argue against a meaningful stimulation of NO generation.

VI. Renal Effects of Leptin

Besides the indirect action of leptin on renal function via sympathoactivation, leptin could exert a direct effect on the kidney. In the rat, leptin receptor expression has been found in the inner zone of the medulla and pyramid, associated with the vascular structures, tubules, and ducts (Serradeil-Le Gal *et al.*, 1997). Several investigators have shown that acute administration of leptin in anesthetized or conscious normotensive lean rats produces significant increases in sodium excretion and urine volume without significant effects on renal blood flow, glomerular filtration rate, or potassium excretion (Jackson and Li, 1997; Villarreal *et al.*, 1998; Beltowski *et al.*, 2002). As expected, these saluretic effects of leptin were blunted in the Zucker rat (Villarreal *et al.*, 2000) but also in high-fat-diet, obese rats (Beltowski *et al.*, 2002). Surprisingly, spontaneously hypertensive rats also were refractory to the diuretic and natriuretic effects of leptin, due perhaps to enhanced renal SNA, because renal denervation restored

the saliuretic response of these rats to leptin (Villarreal *et al.*, 2000). A subsequent study by Shek *et al.* (1998) suggested that the natriuretic action of leptin is not operative at physiological levels because a chronic increase in leptin concentrations within the physiological range in rats did not produce natriuresis, despite an increase in arterial pressure. This study (Shek *et al.*, 1998) provided no support for a significant natriuretic action of leptin and instead suggested that leptin actually may produce a modest antinatriuretic effect (probably due to the activation of the renal SNA) that opposes pressure natriuresis.

However, leptin appears to have an important role in obesity-induced renal damage such as glomerular hyperfiltration and increased urinary albumin loss. In glomerular endothelial cells, leptin stimulates cellular proliferation, transforming growth factor-beta₁ (TGF- β_1) synthesis, and type IV collagen production. Conversely, in mesangial cells, leptin upregulates synthesis of the TGF- β type II receptor, but not TGF- β_1 , and stimulates glucose transport and type I collagen production (Wolf *et al.*, 1999). Chronic leptin treatment induces glomerulosclerosis and proteinuria in normal rats (Wolf *et al.*, 1999). Therefore, leptin may be of relevance to the development of glomerular pathology associated with obesity.

VII. Leptin and Other Cardiovascular Risks

Longitudinal and cross-sectional studies have shown an association between serum leptin concentrations and various cardiovascular risks, including stroke (Soderberg *et al.*, 1999a), chronic heart failure (Leyva *et al.*, 1998; Schulze *et al.*, 2003), acute myocardial infarction (Soderberg *et al.*, 1999b), coronary heart disease (Wallace *et al.*, 2001), and left cardiac hypertrophy (Paolisso *et al.*, 1999). Most of these studies have statistically corrected for differences in body adiposity, though such statistical techniques can be inadequate when there are large differences between groups in confounding variables. Further studies, including animal experimentation, are needed to strengthen and clarify the link between leptin and cardiovascular disease (CVD). The mechanisms by which leptin could contribute to these complications remains unknown. Although the action of leptin in the CNS and the activation of the sympathetic nervous system could play a major role in CVD, the presence of the leptin receptor in the heart suggests that leptin could modulate cardiac function directly (Wold *et al.*, 2002). For example, Nickola *et al.* (2000) have shown a direct effect of leptin on cardiomyocyte contraction that may contribute to altered myocardial function. Interestingly, the cardiac contractile response to leptin was blunted in the spontaneously hypertensive rat (Wold *et al.*, 2002). An effect of leptin on the adenylate cyclase system, the main effector of beta-adrenergic receptors, was demonstrated in a cardiac cell line (Illiano *et al.*, 2002).

VIII. Conclusion

The epidemic of obesity has led to an unwelcome epidemic of CVD, including hypertension. The mechanisms of obesity-related CVD are not fully understood but the discovery of leptin and its effects on the sympathetic nervous system may provide a partial explanation. Leptin has diverse cardiovascular actions, although sympathoactivation is probably the most important. The concept of selective leptin resistance may explain how leptin could contribute to obesity-related hypertension, despite loss of its metabolic effects. Increasing knowledge of the mechanisms and sites of leptin resistance should provide new insights into the pathophysiology of obesity and its treatment.

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