Cardiovascular and Sympathetic Effects of Leptin

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Several studies have shown the association between obesity and hypertension. The pathophysiologic mechanisms of obesity-related hypertension remain unknown. Clinical and experimental studies have shown that obesity is associated with enhanced sympathetic nervous activity. Thus, sympathetic nerve activation seems to play a major role in obesity-associated hypertension. However, the factors responsible for this sympathoactivation have not been identified. Leptin is an adipocyte-derived hormone that promotes weight loss by reducing appetite and food intake and by increasing energy expenditure through sympathetic stimulation to brown adipose tissue. Leptin also produces sympathoactivation to kidneys, hindlimb, and adrenal glands, indicating that the obesity-associated increase in sympathetic nerve activity could be due in part to these sympathetic effects of leptin. However, obesity is associated with leptin resistance, since high circulating levels of leptin were observed in obese subjects. Recent evidences indicate that this leptin resistance could be selective with preservation of sympathetic effects despite the loss of metabolic action of leptin. This suggests divergent central pathways underlying metabolic and sympathetic effects of leptin. Several neuropeptides have emerged as potent candidate mediators of leptin action in the central nervous system, including the melanocortin system, neuropeptide Y, and cortico-trophin releasing factor. A detailed understanding of the multitude and complexity of integrated neuronal circuits and neuropeptide-containing pathways in leptin action will help in understanding the pathogenesis of obesity and related disorders.

Introduction

Obesity is a common disorder associated with diabetes and cardiovascular diseases including hypertension, dyslipidemia, and atherosclerosis [1,2]. An association between obesity and hypertension is supported by epidemiologic studies indicating that high blood pressure is more frequent in overweight subjects compared with lean populations [3]. Experimental studies in humans and animals have shown that weight gain raises arterial pressure and weight loss reduces arterial pressure in both normotensive and hypertensive subjects [4]. Although the pathophysiologic mechanisms of obesity-related hypertension remain under study, some evidence suggest that enhanced sympathetic nervous activity might play a major role in obesity-associated hypertension. Plasma and urinary catecholamines are increased in obese humans as well as in obese animal models [5–7]. Grassi *et al.* [8], using direct measurement with the microneurography method, have shown increased sympathetic nerve activity in obese subjects compared with lean individuals. Pharmacologic blockade of adrenergic activity by a combination of α- and β-adrenergic receptor inhibitors or ganglionic blockade markedly blunts obesity-associated hypertension in dogs fed a high fat diet [10]. Finally, bilateral renal denervation blocks the increase in arterial pressure induced by high fat feeding in dogs [11]. Taken together, these observations indicate the importance of sympathetic nervous system activity in the obesity-associated hypertension in humans and animal models.

Increased sympathetic nervous system activity might result from circulating humoral factors exerting central or peripheral neural action. Insulin is known to increase sympathetic nerve activity. Hyperinsulinemia has been suggested as a link between obesity and hypertension, but experimental studies have challenged this concept [12,13]. Recent evidence indicates that leptin may represent a link between excess weight gain and high arterial pressure through actions on the sympathetic nervous system.

The Concept of Selective Leptin Resistance

Leptin, the product of the gene *ob*/*ob*, is a key afferent signal in the negative feedback loop regulating body weight. This hormone is produced by white adipose tissue and circulates to the hypothalamus to inform the central nervous system about the state of body fat. Leptin regulates body weight and adipose tissue mass by reducing appetite and food intake and by increasing energy expenditure (Fig. 1). Collins *et al.* [14], using norepinephrine turnover, have shown that the increase in energy expenditure is mediated through sympathetic stimulation to brown adipose tissue. Haynes *et al.* [15] from our laboratory used direct measurement of sympathetic nerve activity to

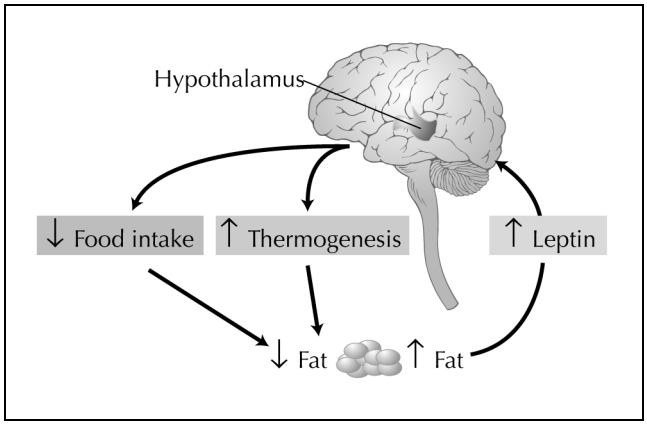

Figure 1. Schematic representation of the loop regulating body weight. Leptin is secreted by the adipocyte and circulates in the blood in concentration proportional to fat mass content. Action of leptin on its receptor present in the hypothalamus inhibits food intake and increases energy expenditure (thermogenesis), leading to decreased adipose tissue mass and body weight.

examine the effects of intravenous administration of murine leptin on sympathetic nerve activity to different tissues in rats. As expected, leptin caused a significant increase in sympathetic nerve activity to brown adipose tissue. Surprisingly, leptin also caused a significant and dose-dependent increase in sympathetic nerve activity to the kidneys, hindlimb, and adrenal glands [15]. This effect of leptin on sympathetic nerve activity to tissues other than brown adipose tissue was unexpected. Others have found that this rise in sympathetic nerve activity induced by leptin was associated with an increase in arterial pressure and heart rate [16].

These findings suggest that leptin contributes not only to the regulation of energy homeostasis, but also to the control of cardiovascular function. This has been confirmed by chronic infusion of leptin that increases arterial pressure and heart rate in conscious rats [17]. Furthermore, agouti obese mice [18] with hyperleptinemia, and transgenic mice overexpressing leptin [19••] have elevated arterial pressure. In contrast, leptin-deficient ob/ob mice have reduced arterial pressure [18]. Thus, leptin appears to contribute to the regulation of cardiovascular function and may be implicated in the pathophysiology of obesity-associated hypertension. In this regard, a significant correlation between blood pressure and plasma leptin concentration in patients with essential hypertension has been described [20].

Numerous studies in humans and animals have shown that plasma leptin levels are significantly elevated in obese individuals relative to lean subjects [21,22]. These findings led to the conclusion that obese subjects are leptin resistant because, despite the high levels of circulating endogenous leptin, they remain obese. Furthermore, exogenous administration of leptin in obese animals produces resistance to the effects of leptin on food intake and body weight [22]. Several mechanisms have been suggested to explain the phenomenon of leptin resistance, such as decreased transport of leptin across the blood-brain barrier [22,23], a defect in the leptin receptor [24], or impaired downstream signaling in the hypothalamus [22,25••]. In addition to multiple mechanisms of leptin resistance, there is emerging evidence that in some models of obesity, leptin resistance may be selective and spare some actions of leptin. For example, in agouti obese mice, there is resistance to the metabolic effects of leptin, but the blood pressure effects appear preserved [19••]. In this regard, we recently demonstrated that agouti obese mice have selective leptin resistance with preservation of the sympathetic actions despite loss of the metabolic effects of systemic leptin. We found that effects of systemic leptin on food intake and body weight were significantly less in agouti obese than in lean controls, whereas the increase in renal sympathetic nerve activity was not different in agouti obese and lean mice across all doses [26]. We subsequently obtained preliminary evidence that selective leptin resistance in these agouti obese mice occurs with central neural as well as systemic administration of leptin, excluding a defect in the transport of leptin to cerebrospinal fluid [27]. This finding of selective leptin resistance has been extended in preliminary studies to other models of obese mice including diet-induced obesity [28]. The concept of selective leptin resistance has potentially important implications because it could help explain how high levels of circulating leptin in obese subjects might contribute to elevated arterial pressure despite resistance to the effects of leptin on appetite and body weight. In support of the persistence of the sympathetic action of leptin in obesity is the finding that plasma leptin concentration correlates significantly with muscle sympathetic nerve activity in obese subjects [29].

Site of Leptin Action in the Central Nervous System

The leptin receptor is a single transmembrane protein belonging to the cytokine receptor super-family. Due to alternative splicing of the mRNA, at least six leptin receptor isoforms have been identified (designated Ob-Ra to Ob-Rf) [30]. Five isoforms (Ob-Ra to Ob-Rd and Ob-Rf) differ in the length of their intracellular domain, while Ob-Re, which lacks the transmembrane domain, is a soluble form of the receptor. The Ob-Rb form encodes the full receptor, including the long intracellular domain that contains all the motifs necessary to stimulate the janus kinases-signal transduction and transcription (JAK-STAT) pathway [31]. STAT proteins stimulate transcription of target genes that mediate some of the cellular effects of leptin. However, some rapid effects of leptin on neuron activity are unlikely to be mediated by a modulation of gene transcription. For example, subsets of hypothalamic neurons were hyperpolarized within minutes of leptin application [32]. Modulation of the activity of mitogen-activated protein (MAP) kinase [33], PI3-kinase [34] type 3 phosphodiesterase [35], IRS protein [33], and

protein kinase C [36] by leptin have been reported and could mediate these rapid effects of this hormone.

Leptin from the plasma is transported to the central nervous system by a saturable, unidirectional system [37] 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 [38]. There is abundant evidence that the hypothalamus is the most important site of leptin action in the brain. The long form of leptin receptor, Ob-Rb, is expressed in several hypothalamic nuclei including the arcuate nucleus, ventromedial hypothalamus, paraventricular nucleus, and dorsomedial hypothalamus [39]. The arcuate nucleus is considered the major site of transduction of the afferent input from circulating leptin into a neuronal response. This is supported by the decrease in food intake induced by local injection of leptin in this area [40], and the inability of central neural administration of leptin to affect food intake [41] or sympathetic nerve activity [42] after the arcuate nucleus has been destroyed. Other brain areas innervated by the arcuate nucleus neurons, such as the paraventricular nucleus and lateral hypothalamus, are considered as downstream neurons of second order in the pathways regulating neuronal activity by leptin [39]. After activation of leptin receptors in the central nervous system, the signal is transduced by a series of integrated neuronal pathways that lead to changes in the nerve activity affecting different functions in the periphery.

Role of the Melanocortin System in Leptin Signaling

There is strong evidence that many of leptin's actions are mediated by stimulation of the melanocortin system. The melanocortins are peptides that are processed from the polypeptide precursor pro-opiomelanocortin (POMC), which is produced by neurons in the arcuate nucleus of the hypothalamus and the nucleus of the tractus solitarius. POMC neurons are known to express the leptin receptor. Leptin binding leads to the secretion of alpha-melanocyte stimulating hormone (α-MSH), which in turn binds to a number of the family of melanocortin receptors (Fig. 2). In the absence of leptin (in ob/ob mice) the expression of the POMC gene is reduced [43]. In the ob/ob mice, injection of leptin significantly increases POMC mRNA expression in the hypothalamus [44].

The critical role for the melanocortin system in leptin signaling emerged with the cloning of melanocortin-4 (MC-4) receptor gene and the demonstration that it is expressed primarily in the brain [45]. Subsequently, it was demonstrated that a synthetic agonist of this receptor (MTII) suppresses food intake, whereas a synthetic antagonist (SHU9119) has the opposite effect [46]. Targeted disruption of the MC-4 receptor induces obesity, and these mice are resistant to both peripherally and centrally administered leptin [47]. Interestingly, heterozygous MC-4 receptor knockout mice are also obese, but less obese than the homozygous knockout mice [47], suggesting an important physiologic role for this receptor. Disruption of both the MC-3 receptor and MC-4 receptor exacerbates the obesity associated with MC-4 receptor deficiency, suggesting nonredundant functions for the two receptors in regulating body weight [48]. The MC-3 receptor appears particularly to influence feeding efficiency and fat storage.

MC-4 receptors also play a role in mediating the effect of leptin on sympathetic nerve activity. Stimulation of hypothalamic MC-4 receptors by central administration of MTII produces a dose-dependent increase in sympathetic nerve activity to brown adipose tissue and kidney that is blocked by the MC-4 receptor antagonist, SHU9119 [49•]. Surprisingly, MC-4 receptor blockade prevents the sympathoexcitatory effects of leptin to the kidneys, but not to brown adipose tissue [49•]. These results suggest that leptin controls sympathetic nerve activity in a tissue-specific manner through different neuronal pathways. The regulatory action of leptin on sympathetic nerve activity to the kidney appears to be mediated by the melanocortin system. We have recent unpublished evidence that renal sympathoactivation to leptin is abolished in homozygous MC-4 receptor knockout mice. Interestingly, we also observed that the maximum increase in renal sympathetic nerve activity induced by leptin in the heterozygous MC-4 receptor knockout mice was the half response of the wild-type mice. These findings confirm the pivotal role of MC-4 receptor in mediating the effect of leptin on renal sympathetic nerve activity.

Further evidence for the importance of the melanocortinergic pathways in leptin signaling has been obtained by studying the syndrome of agouti yellow obesity in mice [50]. These animals have a mutation in the agouti gene that leads to ubiquitous overexpression of agouti protein that functions as an antagonist of MC-1 receptors preventing α-MSH from stimulating melanin synthesis and terminal pigmentation of the hair follicles, leading to the yellow hair [51,52]. Blockade of α-MSH effects MC-4 receptors in the hypothalamus causing obesity in the agouti syndrome. Transgenic mice overexpressing agouti-related protein mimic the critical futures of the obesity syndrome in the agouti yellow mice [53,54]. As with most human obesity, the obesity observed in agouti mice is associated with high levels of circulating leptin [22] and elevated arterial blood pressure [18]. These mice are resistant to the appetite and weight reducing effect of leptin [22], but leptin nevertheless contributes importantly to regulation of arterial pressure in these mice [19••]. The novel concept of selective leptin resistance, discussed previously, may help explain how high levels of leptin in agouti mice contribute to hypertension despite resistance to its metabolic effects.

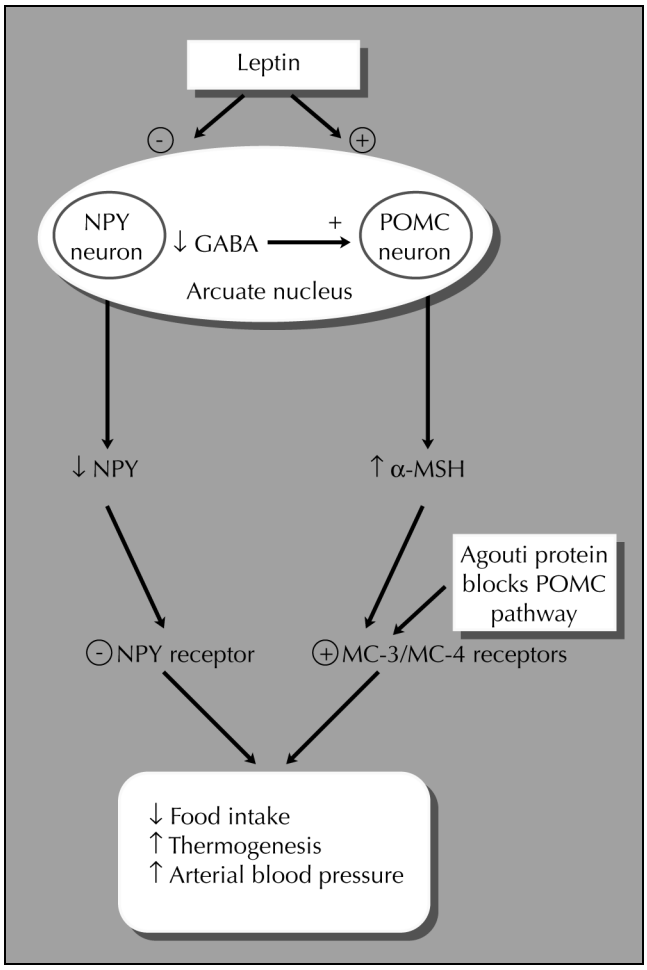

Figure 2. Signaling pathway of leptin in the hypothalamic arcuate nucleus and the interaction of leptin with neuropeptide Y (NPY)- and pro-opiomelanocortin (POMC)-containing neurons. Increased action of leptin inhibits the NPY anabolic pathway and stimulates the POMC catabolic pathway, leading to reduced food intake and increased thermogenesis (resulting in decreased in body weight) and arterial blood pressure (resulting in hypertension). GABA—γ-amino-butyric acid; MC—melanocortin; α-MSH—alpha-melanocyte stimulating hormone.

Interaction of Neuropeptide Y and Leptin in the Hypothalamus

Neuropeptide Y (NPY) has emerged as a candidate mediator of leptin action in the brain after the demonstration of the functionally active, long isoform of the leptin receptor in the NPY neurons [55]. NPY, a 36-amino acid peptide, is the most potent orexigenic (promotes increased energy intake) peptide activated by decreases in leptin [39,56]. In the hypothalamus NPY is synthesized by neurons of the arcuate nucleus and secreted from their terminals in the paraventricular nucleus and lateral hypothalamus (Fig. 2). Injection of NPY into the cerebral ventricles or direct hypothalamic administration increases food intake and promotes obesity [57]. Levels of NPY are dramatically increased in the hypothalamus of leptin-deficient mice [58,59]. Moreover, leptin inhibits NPY gene expression, and knockout of the NPY gene reduces by about 50% the obesity and other endocrine alterations resulting from chronic leptin deficiency in ob/ob mice [60]. Recently, Cowley *et al*. [61•] demonstrated that NPY can also modulate directly the activity of POMC neurons via the release of γ-aminobutyric acid (GABA) (Fig. 2).

Interactions between leptin and NPY in the control of renal sympathetic nerve activity and blood pressure have been described by Matsumura *et al*. [62•]. They found that central neural administration of NPY in rabbits elicited dose-dependent decrease in arterial pressure and sympathetic activity to the kidneys. Pretreatment with intracerebroventricular administration of leptin prevented the depressor and sympathoinhibitory responses to central administration of NPY. This suggests that leptin interferes with NPY action in some manner.

NPY, therefore, has a key role in the control by leptin of body weight, energy homeostasis, and cardiovascular function. Surprisingly, mice in which the NPY gene had been deleted by homologous recombination were phenotypically normal and responded to leptin [63]. This indicates redundant signaling mechanisms, such that in the absence of NPY, leptin acts through other pathways to maintain seemingly normal feeding and body weight regulation. Subsequently, other effector molecules mediating the effects of leptin have been discovered, including melanocortin, orexin, agouti-related protein, galanin, neurotensin, cocaine- and amphetamine-regulated transcript, and corticotrophin releasing factor (CRF) [39,56].

Interaction of Corticotrophin Releasing Factor and Leptin

The complicated nature of leptin signaling pathways may be suggested from the essentially normal phenotype of NPY knockout mice [63], despite the potent stimulatory effects of NPY on food intake and body weight [57]. This suggests that there are complementary and/or overlapping effector systems that compensate the absence of NPY. CRF is a 41-amino acid mammalian neurohormone that inhibits food intake and appears also to mediate leptin actions. Chronic administration of CRF causes sustained anorexia and progressive body weight loss [56,64]. CRF also increases sympathetic activity to brown adipose tissue and kidneys [65,66] in the same pattern observed with leptin [15]. Therefore, we postulated that leptin may induce sympathoactivation through CRF and/or CRF-related neuropeptides. Subsequently, we examined the effects of central administration of CRF on sympathetic nervous activity and the interaction between CRF receptors and leptin in the regulation of sympathetic nerve activity in the rat. We observed that third cerebroventricular administration of CRF produced a substantial dose-dependent increase in sympathetic nerve activity to brown adipose tissue, which was blocked by concomitant central administration of a CRF receptor antagonist (α-helical CRF9-41)

[67•]. We also observed that leptin-dependent sympathetic activation to brown adipose tissue was mediated by the CRF receptor; the sympathoexcitatory effect of leptin to this tissue was substantially inhibited by pretreatment with the CRF receptor antagonist (Fig. 3). These results demonstrate that leptin and CRF or CRF-related peptides interact in the central nervous system to control sympathetic nervous activity to brown adipose tissue [67•]. However, the suppression of the sympathoexcitatory effects of leptin to brown adipose tissue by the CRF receptor antagonist was incomplete, suggesting that other pathways such as neurotensin or orexin may interact with leptin to control sympathetic nerve activity to thermogenic tissue.

Conclusions

Leptin is a key hormone in regulation of food intake and energy homeostasis. Multiple other actions of leptin are potentially relevant not only to control of body weight but also to cardiovascular regulation. Leptin action on the sympathetic nervous system could contribute importantly to obesity-related hypertension because in animal models of obesity the sympathetic action is conserved despite the loss of metabolic effects of leptin.

Modulation of these different functions by leptin is mediated by several neuroendocrine systems including the melanocortin system, NPY, and CRF. A detailed understanding of the multitude and complexity of integrated neuronal circuits and neuropeptide-containing pathways in leptin actions will help in understanding the pathogenesis of obesity and related disorders. For example, these studies may help to identify the molecular basis of selective leptin resistance observed in some models of obesity. Future studies will hopefully determine the precise character and mechanisms of selective leptin resistance.

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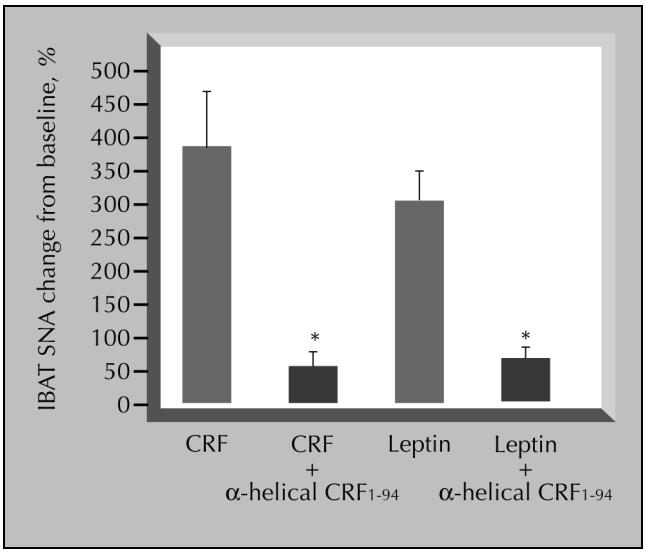

Figure 3. Effect of intracerebroventricular administration of 30 μg of coricotrophin releasing factor (CRF) receptor antagonist (α-helical CRF1-94) on sympathoactivation to thermogenic interscapular brown adipose tissue (IBAT) induced by CRF (5 μg, intracerebroventricularly) or leptin (1000 μg/kg, intravenously) in Sprague-Dawley rats. CRF receptor antagonist substantially attenuated increases in sympathetic nerve activity (SNA) to IBAT induced by CRF or leptin (*$P < 0.001$).

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