



PAPER

Hypothalamic pathways underlying the endocrine, autonomic, and behavioral effects of leptin

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Leptin affects body weight by decreasing food intake, activating the sympathetic nervous system and regulating neuroendocrine function. This type of regulation is a hallmark of hypothalamic control, which typically integrates autonomic, endocrine and behavioral responses. We have performed a series of experiments investigating hypothalamic pathways underlying these actions of leptin. We found that leptin activates neurons that coexpress pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) mRNA. These neurons innervate several sites, including sympathetic preganglionic neurons in the spinal cord, neurons in the paraventricular hypothalamic nucleus (PVH), and melanin-concentrating hormone and orexin neurons in the lateral hypothalamic area (LHA). Following leptin administration, POMC neurons express both Fos and suppressor of cytokine signalling-3 (SOCS-3) mRNA. In contrast, leptin induced SOCS-3 expression in neuropeptide Y (NPY) neurons but not Fos, suggesting that leptin acts differentially on NPY and POMC cells. We also investigated potential downstream targets of leptin responsive NPY and POMC neurons by assessing the distribution of the melanocortin 4 receptor (MC4-R) mRNA and Y1 and Y5 NPY receptor mRNA in chemically defined neurons. We found dense MC4-R mRNA expression in several sites including the PVH and LHA. Using dual-label *in situ* hybridization we found that MC4-R mRNA is coexpressed in PVH cells expressing pro-TRH mRNA. We also found Y1 and Y5 NPY receptor mRNA in the PVH in patterns very similar to that of MC4R, suggesting that these receptors may be coexpressed on at least some PVH neurons. These results provide a neuroanatomic framework explaining the endocrine, autonomic and behavioral effects of leptin.
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Introduction

The identification of the *ob* gene greatly increased the understanding of the molecular control of metabolism and body weight.¹ Leptin, the product of the *ob* gene, is a circulating hormone produced by white adipose tissue that has potent effects on feeding behavior, thermogenesis, and neuroendocrine responses.^{2–6} The severe obesity caused by leptin absence in rodents and humans makes it clear that leptin is a fundamental hormone regulating energy homeostasis. It is now established that the hypothalamus integrates leptin signals into a coordinated endocrine, behavioral and autonomic responses that maintain homeostasis. This brief

review describes experimental evidence regarding the central neural pathways that may mediate the effects of leptin.

Leptin-responsive cell groups

Several studies have demonstrated that long form leptin receptors are expressed in several hypothalamic nuclei with the highest concentration in nuclear groups of the ventrobasal hypothalamus.^{7–10} In our studies, we found within the hypothalamus dense hybridization in the arcuate, dorsomedial, ventromedial and ventral premammillary nuclei. Within the dorsomedial nucleus (DMH), particularly dense hybridization was observed in the ventral and caudal regions of the nucleus. Hybridization was observed throughout the arcuate nucleus extending from the retrochiasmatic region to the posterior periventricular region. Moderate hybridization is also observed in the periventricular hypothalamic nucleus, lateral hypothalamic area, medial mammillary nucleus and posterior hypothalamic nucleus.⁸

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Table 1 Examples of hypothalamic integration underlying leptin action; a proposed model of the central neural pathways that mediate the multiple effects of leptin

Type of response	Example	Proposed pathway	Relevant references
Endocrine	Regulation of proTRH mRNA and thyroid hormone levels	Arc (POMC/CART and NPY/AgRP neurons)→PVH (TRH neurons)	17,38,39,52,37,53
Autonomic	Activation of body temperature, energy expenditure, and blood pressure	Arc (POMC/CART neurons)→IML (sympathetic preganglionic neurons)	36,54,55
Behavioral	Inhibition of food intake	Arc (POMC/CART and NPY/AgRP neurons)→LHA (MCH and ORX neurons)	14,46,48,49

Arc, arcuate nucleus of the hypothalamus; AgRP, agouti related protein; CART, cocaine- and amphetamine-regulated transcript; IML, interomedial lateral cell column; LHA, lateral hypothalamic area; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; PVH, paraventricular nucleus of the hypothalamus; pro-TRH, pro-thyrotropin releasing hormone.

The distribution of leptin receptors in the hypothalamus supports the hypothesis that the hypothalamus is the major site of action underlying leptin's physiological effects. We and others have investigated induction of immediate early genes by leptin in brain nuclear groups. We first used expression of *c-fos* as a generic marker of neuronal activation. We found that intraventricular administration of leptin activates (induces the expression of Fos-immunoreactivity) several nuclear groups in the rat brain including the ventromedial, dorsomedial, and paraventricular hypothalamic nuclei.^{11–13}

We did not find leptin-activated neuropeptide Y (NPY) neurons in the medial subdivisions of the arcuate nucleus of the hypothalamus.¹⁴ At first glance, this may seem paradoxical as the arcuate nucleus contains dense concentrations of leptin receptors and NPY neurons regulate feeding.¹⁵ However, since NPY is thought to increase feeding in this pathway, we predicted that these neurons would be inhibited by leptin^{7,16,17} and thus would probably not express Fos. However, we did find leptin activated cells in the lateral edges of the arcuate in neurons containing pro-opiomelanocortin (POMC). Activation of these neurons is consistent with data suggesting that leptin positively regulates POMC expression.^{18–20}

Recently, we identified a leptin-induced inhibitor of leptin-signal signaling.²¹ This inhibitor is a member of the suppressors of cytokine signaling (SOCS) family. SOCS proteins are rapidly induced following activation of type I cytokine receptors that utilize the JAK tyrosine kinase signaling pathway. SOCS proteins are intracellular regulators that feedback to inhibit STAT phosphorylation induced by cytokine receptor activation.^{22–24} The long form of the leptin receptor is a member of the type I cytokine receptor family,²⁵ thus we hypothesized that leptin may induce one or more SOCS family members. We found that SOCS-3 mRNA is induced following leptin administration in a pattern very similar to that of the long form of the leptin receptor mRNA.²¹ Thus, we propose that SOCS-3 expression is a better marker than Fos (or other immediate early genes) for direct leptin action in the brain. As discussed above, NPY neurons do not express leptin-induced Fos. However, NPY

neurons do express SOCS-3 mRNA after leptin administration demonstrating that leptin directly acts on NPY neurons.¹⁴ The lack of Fos and the presence of SOCS-3 expression by NPY neurons suggests that the leptin receptor has been engaged, but NPY neurons may be inhibited by leptin.

The importance of the melanocortin systems in regulating feeding is now established. For example, targeted deletion of the melanocortin 4 receptor (MC4-R) gene results in obesity.²⁶ The CNS agonist of MC4-Rs is α -MSH, a derivative of the pro-opiomelanocortin (POMC) precursor.²⁷ POMC neurons are found in the lateral part of the arcuate nucleus²⁸ and leptin receptor mRNA is coexpressed in a very high percentage of these arcuate nucleus neurons.²⁹ Furthermore, in leptin-deficient *ob/ob* mice or in fasted rodents (when leptin levels rapidly fall) POMC mRNA is markedly reduced. This decrease in POMC is blocked by exogenous leptin administration.^{18–20} In addition, the leptin effects of decreasing food intake and activating the sympathetic nervous system can be blunted by melanocortin receptor antagonist co-administration.³⁰

The role played by hypothalamic arcuate nucleus POMC neurons in regulating feeding has been reinforced by the demonstration that CART (cocaine and amphetamine responsive transcript)³¹ regulates food intake. CART neurons are found throughout the CNS, including the arcuate nucleus and the retrochiasmatic area.^{32,33} Injections of CART peptide into the lateral ventricle suppress feeding.^{34,35} CART mRNA expression is regulated by leptin. Like POMC, CART mRNA is reduced in the arcuate nucleus in leptin-deficient *ob/ob* mice and fasted rats. Leptin administration normalizes arcuate CART mRNA levels. Thus, hypothalamic CART systems may participate in central regulation of feeding and body weight.³⁵ Consistent with this hypothesis, we found that leptin activates CART neurons in the retrochiasmatic area and lateral arcuate nucleus. We also found that CART neurons in the RCA and the Arc coexpress POMC mRNA.³⁶

CART peptide-immunoreactive terminals innervate central autonomic sites, including the sympathetic preganglionic neurons in the spinal cord.³³ Injection of retrograde

tracer into the spinal cord demonstrated that the CART inputs to the spinal cord originate in part from neurons in the lateral part of the arcuate nucleus and the adjacent retrochiasmatic nucleus.³⁶ Furthermore, combining these markers with leptin administration demonstrates that there is leptin-induced Fos expression in a high proportion of CART neurons in the arcuate nucleus and the retrochiasmatic area that innervate the sympathetic preganglionic cell column.³⁶

Leptin activates neurons Innervating the PVH

The effects of leptin on the hypothalamo-pituitary-adrenal (HPA) and thyroid axes illustrates that the paraventricular nucleus of the hypothalamus (PVH) is involved in mediating several of the effects of circulating leptin as this nucleus contains hypophysiotropic thyrotropin releasing hormone (TRH) and corticotropin releasing hormone (CRH) neurons. In our initial studies we found that leptin administration induced Fos-IR within the parvocellular subnuclei in the PVH.¹¹ The activation of the PVH by leptin could occur directly as low levels of leptin receptors are present in the PVH^{8,37} and leptin induces SOCS-3 mRNA in a very similar pattern in the PVH.²¹ However, leptin receptors are found in the highest densities in the ventrobasal hypothalamus in regions known to project to the PVH. In addition, the effects of leptin to regulate the thyroid axis and pro-TRH mRNA expression^{17,38} require an intact arcuate nucleus of the hypothalamus.³⁹ Therefore, we hypothesized that leptin action within the PVH is due at least in part to innervation from leptin responsive neurons in the medial basal hypothalamus. We investigated the distribution of leptin-activated projections (Fos containing cells) to the PVH.¹² We injected the retrograde tracer, cholera toxin B subunit (CTb) into the PVH, followed by i.v. injections of leptin. We found leptin-activated PVH-projecting neurons in the lateral arcuate nucleus and in the dorsomedial hypothalamic nucleus (DMH).¹² In preliminary experiments we have identified some of these leptin-activated cells in the arcuate nucleus as POMC neurons.

We also investigated the potential downstream targets of leptin responsive NPY and POMC neurons by assessing the distribution of the melanocortin 4 receptor (MC4-R) mRNA and Y1 and Y5 NPY receptor mRNA in chemically defined neurons in the PVH. We found dense MC4-R mRNA expression in several sites including the PVH. Using dual-label *in situ* hybridization we have found that MC4-R mRNA is coexpressed in PVH cells expressing pro-TRH mRNA.³⁷ We also found Y1 and Y5 NPY receptor mRNA in the PVH in patterns very similar to that of MC4R, suggesting that these receptors may be co-expressed on at least some PVH neurons. These findings suggest that TRH neurons in the PVH are direct targets of leptin responsive POMC and NPY neurons in the arcuate nucleus. This hypothesis is consistent with the recent findings of Cowley and colleagues. They used electrophysiology and a hypothalamic slice model and found that

α -MSH and NPY have antagonistic effects on PVH neurons,⁴⁰ although this action may be due to presynaptic interactions with inhibitory interneurons rather than direct postsynaptic mechanisms. Nonetheless, it is likely that some of the effects of leptin are due to the projection of leptin-responsive neurons in the arcuate nucleus to PVH neurons.

Downstream targets of the arcuate nucleus: the LHA

Multiple pathways are likely engaged by circulating leptin to regulate feeding behavior. Included are diffusely-projecting pathways arising in the lateral hypothalamic area (LHA) that innervate the cerebral cortex and may play a role in the complex regulation of feeding behavior and hunger. The LHA has long been considered a region regulating food intake as lesions of this region result in decreased food intake and body weight.⁵ The LHA contains neurons that innervate the entire neuraxis including monosynaptic projections to several regions of the cerebral cortex. Melanin-concentrating hormone (MCH) and orexin neurons (ORX; also called hypocretin)⁴¹⁻⁴⁴ are included in these projections. Central injection of MCH and ORX increase food intake in the rat.^{42,45} In addition, targeted deletion of the MCH gene results in hypophagia and leanness.⁴⁶

Leptin receptors are present in the LHA; however, the densest collections of leptin receptors are found in medio-basal hypothalamic cell groups including POMC²⁹ and NPY⁴⁷ neurons. We investigated the link between leptin regulated neurons in the arcuate nucleus and MCH and ORX neurons in the LHA. We examined the AgRP and α -MSH-immunoreactive axons in the lateral hypothalamic area and found an intense innervation of both MCH and ORX neurons.^{48,49} Both sets of inputs probably come from the arcuate nucleus, because it is the only source of AgRP in the brain, and α -MSH-containing neurons are likewise found in a very restricted distribution. Recent studies show nearly complete colocalization of NPY and AgRP in the arcuate nucleus.^{49,50} Earlier experiments demonstrated that NPY injections increased feeding when injected into the paraventricular nucleus and the region immediately lateral, which contains the perifornical MCH and ORX neurons.⁵¹

To test the functional significance of this pathway by leptin we investigated the distributions of leptin-regulated neurons projecting to the LHA.¹⁴ We identified leptin-sensitive neurons in the arcuate nucleus that innervate the LHA using retrograde tracing with leptin administration. We found retrogradely labeled cells in the Arc contained NPY and POMC mRNA. Following leptin administration, NPY cells in the Arc did not express Fos, but expressed SOCS-3 mRNA. In contrast, leptin induced both Fos and SOCS-3 expression in POMC neurons, many of which also innervated the LHA. These findings suggest that leptin differentially engages NPY and POMC neurons that project to the LHA, linking circulating leptin and neurons that regulate feeding behavior.

Conclusions

There has been tremendous recent progress in understanding the central pathways that underlie the physiological effects of leptin (Table 1). In addition, the molecular basis for several obesity syndromes is now understood. It is clear that leptin interacts with hypothalamic systems to regulate endocrine and autonomic function. CART and POMC are co-expressed in the arcuate nucleus of the hypothalamus and represent anorectic neuropeptides that are positively regulated by leptin. Medial arcuate NPY/AgRP neurons are negatively regulated by leptin. Both populations of arcuate neurons provide a major source of input to the paraventricular hypothalamic nucleus and the lateral hypothalamic MCH and ORX systems. Moreover, the lateral arcuate α -MSH neurons contain CART and project to sympathetic preganglionic neurons in the spinal cord that are activated by leptin and may mediate leptin effects on autonomic responses including energy expenditure and blood pressure. The regulation of these antagonistic populations by leptin and the projections of these cells to the LHA, the paraventricular hypothalamic nucleus, and to pools of autonomic preganglionic neurons in the medulla and spinal cord is likely to be critical in the regulation of endocrine, autonomic, and behavioral effects of leptin.

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