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Leptin participates in the regulation of glucocorticoid and growth hormone axes

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An adipocyte factor transducing the quantity of adipose to a hormonal message that targets the hypothalamus has been postulated for many years. Recently, a gene that encodes such a protein (named leptin*) was identified. These findings indicate that adipose tissue performs important biological functions beyond storage of fat. In fact, data indicate that adipose tissue functions very much like other endocrine tissues, releasing a hormone into the circulatory system to relay a message to its target. Although there may be more than one target tissue for leptin, the best accepted component of any leptin endocrine axis is the hypothalamus. Because this integrative center is fundamental to many neuroendocrine axes, classical endocrine feedback circuits must now be extended to include leptin. Leptin deficiency is extremely rare and has been only reported in a mutant morbid obese mouse strain and in two children who also suffer from extreme obesity. Most types of human and murine obesity are accompanied by elevated leptin levels, and the concentrations of this hormone correlate well with degree of adiposity. Thus, it is thought that leptin may serve as a fuel gauge for the hypothalamus. When sufficient fuel is present or when lipid is being synthesized and stored in adipose, leptin levels rise and reduce further feeding behavior. In addition, a signal of abundant fuel reserve also serves as a message that energy can be expended for anabolism of protein and other biological processes such as growth, repair, and reproduction. Our data, together with those reported by others, indicate that leptin inhibits the hypothalamic-pituitary-adrenal axis (HPAA) and thus antagonizes the catabolic glucocorticoids. A review of available data also supports that leptin stimulates the hypothalamic-growth hormone axis and hence promotes protein synthesis. Energy must be expended to support this anabolic state, and data indicate the energy is derived from metabolism of adipose reserve. Such loss of lipid would tend to decrease plasma leptin levels and thereby signal a state for replenishment of adipose stores.* (J. Nutr. Biochem. 9:553–559, 1998) *© Elsevier Science Inc. 1998*

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Introduction

Anatomical recognition of organs that were proposed to release active substances were described from about 1500 to 1800. Ernest Starling and William Bayliss conceived the endocrine system in 1904 as a complex of chemical messages coordinating the functions of different tissues. By 1930, hormones from the pituitary, gonads, thyroid, parathyroid, adrenal, and pancreas were identified and often used therapeutically to treat endocrinopathies. During the next four decades, new hormones were isolated from these endocrine organs and a new tissue, the hypothalamus, was proven to be a component of the endocrine system. In 1994, adipose tissue became the newest member of this endocrine tissue family.

An adipocyte factor that transfers a hormonal message to the hypothalamus has been postulated for many years. Coleman[1] demonstrated in 1973 that $Lepr^{db}/Lepr^{db}$ (formerly *db/db*) mice were resistant to such a circulating satiety factor, and thus the mice were hyperphagic, diabetic, and obese. Further, Lep^{ob}/Lep^{ob} (formerly *ob/ob*) mice were sensitive to this satiety factor but apparently did not secrete

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it. At the end of 1994, a gene that encodes this protein was identified by Friedman's group.[2] Adipose tissue is the major site for expression of this gene. The 16-kD gene product was named *leptin*, derived from the Greek root "leptos," meaning "thin."[3] Administration of recombinant leptin to Lep^{ob}/Lep^{ob} mice corrects their obesity and associated endocrinopathies.[3–5] However, similar treatment of $Lepr^{db}/Lepr^{db}$ mice, which suffer from a leptin receptor mutation, is ineffective.[3,4]

These findings suggest that adipose tissue performs important biological functions beyond storage of fat. In fact, adipose tissue functions very much like any other endocrine tissue, providing a change in circulating leptin that is perceived by the brain as a signal of lipid storage level. To fully satisfy requirements for a hormone, evidence that leptin is secreted from adipocytes[6] into blood of rodents[7] and human[7,8] was presented. The major target for this hormonal message appears to be the hypothalamus. Direct injection of leptin into the lateral cerebral ventricle of Lep^{ob}/Lep^{ob} mice generated a potent anorectic effect that could only be matched by much higher doses of the protein when it was administered subcutaneously.[9]

Tartaglia and colleagues[10] observed that leptin bound to choroid plexus and identified the leptin receptor gene (Lepr) by expression cloning. Others[11–13] used genetic mapping and genomic analysis to search for a mutation in the Lepr gene. This receptor closely resembles the class I cytokine receptor family and has at least five spliced isoforms in the mouse (four in human). The full-length leptin receptor has a long intracellular domain that is thought to be crucial for signal transduction[14] and is only located in the hypothalamus. A single-point mutation in this gene results in abnormal splicing of the receptor, which does not contain an intracellular region and is proposed to be incapable of signal transduction, leads to leptin resistance, and is likely the cause of severe obesity and diabetes reported in $Lepr^{db}/Lepr^{db}$ mice.[11,14,15] Spliced variant isoforms of the Lepr are located in many tissues including the hypothalamus, choroid plexus, heart, lung, liver, skeletal muscle, and kidney, but the abundance of these molecular forms is extremely low.[16] Functions for these variants are unknown but may involve transport of leptin from blood to its target cells.[17,18]

Thus, leptin fulfills Starling's definition of a hormone. It is carried from adipose tissue, where it is secreted, to the hypothalamus by means of the circulatory system, and the continually recurring storage and dissipation of lipid determine its production and secretion.

The leptin message

Development of sensitive immunoassays permits measurement of leptin during different physiological states. Studies in men and women clearly indicate that circulating leptin values are positively correlated with quantity of body fat (reviewed in Caro et al., 1996).[19] Further, fasting inhibits and refeeding stimulates leptin secretion in rodents[20,21] and human,[22] suggesting that the leptin signal is one of fuel storage or energy availability. Indeed, a rapid decrease in energy stores resulting from short periods of intense exercise is associated with decreased plasma leptin levels.[23,24] Further, leptin levels are dramatically reduced following severely prolonged negative energy balance that is observed in malnutrition of patients suffering from anorexia nervosa.[25,26] Only in rare cases of leptin gene mutation reported in a strain of mice[2] and two related girls[27] are extremely low levels or undetectable concentrations of leptin associated with overnutrition and obesity. Thus, circulating levels of leptin function like a fuel gauge, broadcasting the level of energy storage to brain and perhaps other tissues. In exceptional cases of leptin mutations, the lack of such a signal results in compensatory mechanisms to continuously replenish the lipid reserve and, consequently, is the etiology of severe obesity.

Hypothalamus is the principal leptin target

Leptin's message is received by Lepr in several hypothalamic nuclei including the arcuate, ventromedial, dorsomedial, and lateral hypothalamic nuclei.[16] Only the full-length receptor can transduce the leptin hormonal signal to a neuronal directive.[14] In the arcuate nucleus, leptin reduces the excitatory input to neuropeptide-Y (NPY) neurons and produces inhibitory postsynaptic effects[28] that rapidly decrease NPY release.[9] Long-term leptin signaling results in decreased NPY mRNA expression.[9,29]

Considerable evidence advocates NPY neurons of the arcuate nucleus as a major center for regulating fuel homeostasis and its associated adaptive endocrinology. These NPY neurons project to other areas of the hypothalamus that regulate feeding behavior and coordinate neuroendocrine secretion.[30] Careful mapping of NPY-stimulated feeding has been reported.[31] Fasting and starvation are powerful stimuli, while refeeding is inhibitory to these neurons and their synapses. In concert, hormones responsible for metabolic homeostasis are regulated by NPYergic transmission. Activation of NPY neurons is associated with increased insulin secretion,[32] inducement of the hypothalamic-pituitary-adrenal axis,[33] as well as inhibition of both the thyrotropin-releasing hormone-thyroid axis[34] and the hypothalamic-growth hormone axis. In addition, both episodic basal and cyclic release of luteinizing hormone-releasing hormone (LHRH) are stimulated by NPY, rendering appropriate output from these neurons essential for fertility.[35,36]

While leptin signaling in the arcuate nucleus is inhibitory, neurons in the ventromedial, dorsomedial, and ventral premammillary hypothalamic nuclei appear to be activated by leptin.[37,38] These neurons may release satiety neuropeptides such as cholecystokinin and cortiocotropin-releasing hormone (CRH) that are also important hypothalamic components of the sympathetic nervous system[39,40] and that participate in regulation of body temperature, resting energy expenditure gastrointestinal motility, and insulin secretion.

The hypothalamus is an important central processing center that integrates neuroendocrine circuits, and it depends on leptin afferent information to extend some or all neuroendocrine feedback loops, especially those that are dependent and differentially regulated by fuel reserve level.

The hypothalamic–pituitary–adrenal–adipose axis (HPAAA)

Much endocrinology has been learned by studying the consequences following disconnection or surgical removal of an endocrine gland. Although adipose tissue ablation is not practical, leptin was discovered because of the obese phenotype resulting from mutations in the leptin gene.[2] These leptin-deficient mice are characterized by high expression of hypothalamic NPY[41] as well as increased levels of feeding, plasma corticosterone, and plasma insulin, suggesting that leptin is inhibitory to NPY neurons (discussed above) as well as the HPAA.

CRH neurons comprise much of the complex hypothalamic paraventricular nucleus (PVN), which is divided into neurons that project to the median eminence[42,43] and neurons that give rise to descending efferent autonomic transmission.[44] Interestingly, induction of Fos protein after acute i.v. leptin administration was detected in only these latter autonomic regions of the PVN.[37] Fos immunoactivity was not noticeably altered in the medial subneurons that contain CRH, project to the median eminence, and participate in regulation of the HPAA. Further, we have recently demonstrated that leptin inhibits hypoglycemia-mediated CRH release in vitro and stress-stimulated HPAA in vivo.[45] Hypoglycemia and restraint were techniques used to stimulate the PVN hypophysiotropic CRH neurons. Some studies have failed to appreciate the different subdivisions of the PVN; as a consequence, they have measured expression of CRH mRNA in the entire anatomical complex. The level of this transcript in the PVN of Lep^{ob}/Lep^{ob} mice, which should be most sensitive to the adipocyte hormone, is not altered by leptin treatment,[29] but this same group has reported that administration of leptin to Long-Evans rats increases CRH message levels in this complex nucleus.[46]

In addition, unstimulated hypothalamic slices[47] or hypothalamic explants[48] appear to release CRH after exposure to leptin. Collectively, these data support that leptin negatively feeds back at the hypothalamic level to inhibit HPAA. Leptin also stimulates the CRH-sympathetic neuronal network, which is distinct from the HPAA. Experimental designs that measure cumulative changes in CRH release or mRNA expression in the basal state likely reflect stimulation of the latter. Decreased CRH release or mRNA expression by the medial subneurons of the PVN can best be observed under conditions of HPAA activation such as in Lep^{ob}/Lep^{ob} mice, during fasting, or during stress.

The etiology of obesity observed in Lep^{ob}/Lep^{ob} mice involves failure to produce functional leptin,[2] and replacement of leptin by exogenous administration to Lep^{ob}/Lep^{ob} mice corrects the hypercorticism.[9] Rapid frequent blood sampling of six healthy men permitted the measurement of leptin, ACTH, and cortisol for 24 hr.[49] These data clearly demonstrate that plasma leptin concentrations are inversely correlated to both plasma ACTH and plasma cortisol levels, supporting an inhibitory role for leptin on the HPAA (*Figure 1*). Prolonged fasting, which activates the HPAA, is accompanied by decreased endogenous leptin secretion. The activated HPAA during fasting, however, is attenuated by administration of exogenous leptin.[50] In addition, elevated plasma ACTH and corticosterone levels resulting from restraint stress are blocked by administration of exogenous leptin.[45]

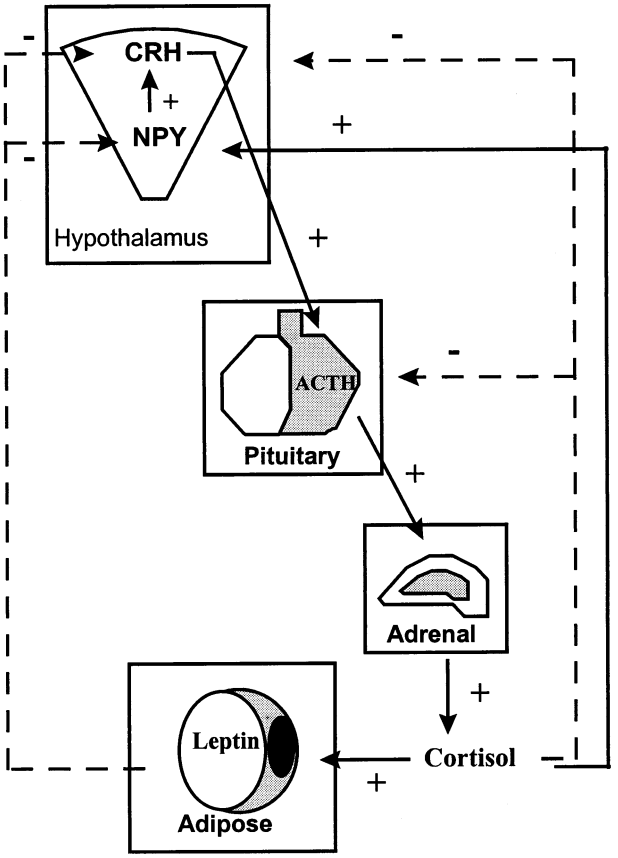

Figure 1 The hypothalamic–pituitary–adrenal–adipose axis. Within the hypothalamus, neuropeptide-Y (NPY) stimulates (+) corticotropin-releasing hormone (CRH) release into the hypothalamic–hypophyseal portal circulation. In turn, CRH stimulates the pituitary gland to synthesize and release adrenocorticotropic hormone (ACTH), which stimulates glucocorticoid production by the adrenal cortex. This steroid stimulates leptin secretion from adipose tissue, which feeds back to inhibit (−) hypothalamic release of NPY and (or) CRH. Negative feedback control of CRH- and ACTH-release is also provided by cortisol (glucocorticoid). In opposition, cortisol stimulates NPY synthesis and release.

Leptin actions at the pituitary level of the HPAA are unclear. Full-length leptin receptor mRNA has been detected in the adenohypophysis of mice[47] and sheep,[51] but these investigators could not differentiate which cell type expresses this receptor. Mouse pituitary slices in perifusion release ACTH (1.5–2-fold) acutely when incubated with leptin.[47] We also found a slight (1.4-fold) but not significant stimulation of ACTH release from rat primary cultured pituitary cells when incubated with leptin.[45] In contrast, we observed almost a 9-fold increase in CRH-mediated secretion of ACTH that was not altered by the co-administration of leptin. It is unfortunate that Raber and colleagues[47] did not compare leptin stimulation to that observed by CRH. Because leptin appears to inhibit the HPAA, we do not believe that leptin significantly alters secretion of ACTH by corticotropes. Obviously, further investigation will determine the specific cell type(s) that express leptin receptors in the pituitary.

Although there is no demonstration of leptin receptors in the adrenal cortex, leptin has been reported to inhibit cortisol release from primary cultured bovine adrenal cortex cells.[52] Further studies are needed to confirm this observation in other species.

Cortisol, however, stimulates leptin secretion,[6,53,54] and this finding permits insertion of leptin in feedback regulation of the HPAA (*Figure 1*). Physiological states of low fuel supply such as fasting are characterized by low circulating leptin levels. In turn, plasma ACTH and glucocorticoid levels are stimulated by increased CRH release into the median eminence. It is important to determine whether decreased leptin levels disengage its inhibition of CRH release directly or indirectly by freeing leptin inhibition of NPY-stimulated CRH release.[33,55] Finally, elevated glucocorticoid stimulates leptin secretion to complete this negative feedback axis. It must be remembered that corticosterone has also been demonstrated to stimulate hypothalamic NPY levels.[56] Therefore, in the rare cases of leptin deficiency, the hypothalamic-pituitary-adrenal axis is intact with negative feedback exerted by glucocorticoid at both the hypothalamus and pituitary, but nevertheless this axis remains activated. These elevated glucocorticoid levels result in overexpression of NPY, and thus obesity that is corrected by leptin replacement.

The hypothalamic–growth hormone–adipose axis

Growth hormone (GH) levels are suppressed in leptin-deficient male Lep^{ob}/Lep^{ob} mice.[57] Unfortunately, it is still unknown whether exogenous leptin replacement therapy would rescue the depressed hypothalamic-GH axis of Lep^{ob}/Lep^{ob} mice. However, exogenous leptin administration does rescue fasting-induced depression of GH secretion in normal rats,[58,59] which is associated with decreased secretion of endogenous leptin.[59] Thus, it appears that leptin is stimulatory to the hypothalamic–GH axis.

Secretion of GH is regulated by complex interaction of neural and hormonal feedback systems that result in a striking pulsatile pattern. Extensive evidence indicates that this episodic pattern of GH release is produced by an interchange between at least two hypothalamic hormones; a GH-releasing hormone (GHRH) and an inhibitory hormone, somatostatin (SRIF).[60,61] Recently, we learned that leptin inhibits SRIF mRNA expression and SRIF release.[62] It is important to determine whether such inhibition is direct or a consequence of leptin inhibition of NPY release[9] because NPY neurons stimulate release of SRIF.[63] Differentiation of the precise pathway awaits appropriate selective NPY antagonists.

A major outcome of GH stimulation is to induce production of insulin-like growth factor-I (IGF-I), and anabolic actions of GH may be mediated through IGF-I.[64] It is still debatable if GH-mediated production of IGF-I has local autocrine and paracrine physiology or whether the peptide is secreted into the circulation and functions as a hormone. In fact, significant local stimulation of IGF-I may leak into the general circulation to function as a hormone. Although IGF-I is produced by most organs, the liver is the major source of the circulating peptide.[65] Circulating IGF-I feeds back to inhibit GH release at both the pituitary[66] and hypothalamus[67,68] to complete this neuroendocrine feedback loop.

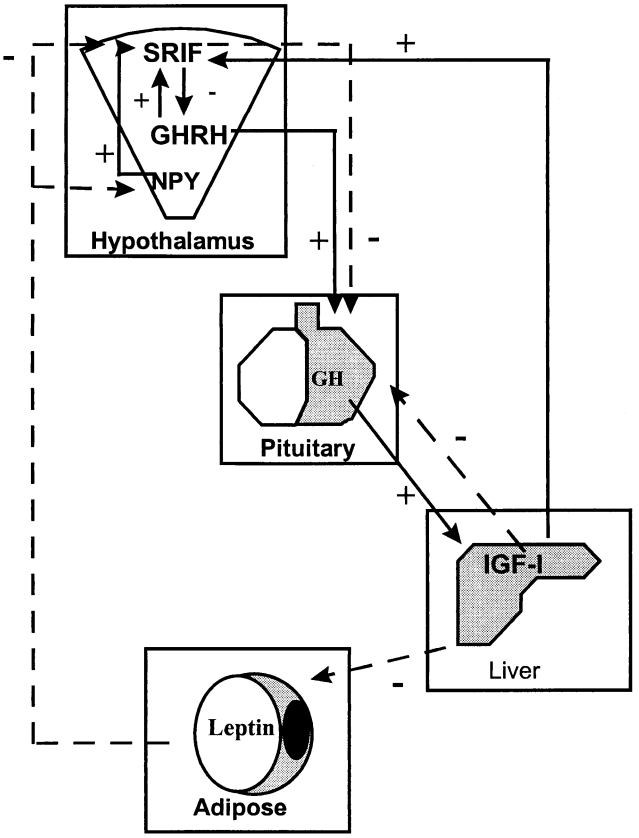

Figure 2 The hypothalamic–GH–IGF-I–leptin axis. Growth hormone-releasing hormone (GHRH) stimulates (+) and somatostatin (SRIF) inhibits (−) growth hormone (GH) release by the pituitary gland. GH stimulates the liver to release IGF-I into the circulation where it can inhibit release of leptin from the adipose tissue and thus disengage leptin-mediated inhibition of hypothalamic SRIF release. IGF-I feeds back to inhibit pituitary GH release directly and indirectly by stimulating SRIF release.

IGF-I also appears to inhibit leptin gene expression by rat adipose tissue.[69,70] Such decreased circulating leptin would release its inhibition of SRIF secretion and therefore augment IGF-I inhibition of GH secretion. These data indicate that the hypothalamic–GH–IGF-I axis should be extended to include leptin (*Figure 2*). Indeed, GH-deficient adults present depressed circulating IGF-I levels and elevated plasma leptin concentrations that are corrected by GH replacement therapy.[71,72]

Recent clinical observations and experimental animal data clearly indicate that regulation of this neuroendocrine axis is substantially impacted by nutrition.[73] Fasting and disorders of nutritional deprivation and malnutrition in humans are associated with elevated serum GH and depressed IGF-I concentrations.[74,75] Caloric deprivation in rat, however, practically abolishes GH release.[59,76] In both species, the magnitude of IGF-I reduction relates to the severity of nutritional insult, and IGF-I levels increase with nutritional rehabilitation.[73] Therefore, changes in circulating IGF-I levels are positively correlated with changes in blood leptin levels and, in concert, supply a signal of fuel

reserve. Additional studies will elucidate if leptin directly stimulates circulating IGF-I levels.

Decreased leptin signals may predominate over lack of IGF-I feedback in rat and, consequently, GH secretion is blunted during fasting. Indeed, pulsatile GH secretion, but not plasma IGF-I levels, is rescued by exogenous administration of leptin.[59] Conversely, in humans, decreased IGF-I negative feedback could override the depressed leptin signal and thus present a stimulus for GH secretion. These apparent species differences in nutritional regulation of the hypothalamic–GH–IGF-I–leptin axis may be a consequence of data obtained at different states of growth and body adipose content rather than actual species differences. Most studies in rat utilize relatively young growing rats that are very lean and are fed a balanced low-calorie-dense diet. Studies in humans typically employ young adults of average body composition who consume a diet that has greater caloric density. Further studies are needed in both the human and the rat to better characterize regulation of GH secretion during nutritional deprivation.

GH secretion is impaired in obese Zucker rats. Because this obese genotype is relatively insensitive to leptin (see above), it follows that SRIF release from hypothalamus of obese Zucker rats is increased.[77] Such leptin-resistant increases in SRIF secretion result in decreased plasma GH.[78] Further, the decreased GH stimulation of IGF-I may be countered by nutritional stimulation of IGF-I. Thus, IGF-I levels do not change during overnutrition.[77] In a similar manner, most phenotypes of human obesity are associated with reduced GH levels and either decreased or normal IGF-I levels.[73] Future studies employing exogenous leptin administration or leptin-sensitivity enhancers will determine whether reduced GH levels observed in obesity can be rescued by activation of the leptin component of this axis.

Conclusion

Adipose tissue sends a message of fuel supply to the hypothalamus by secreting the hormone leptin to achieve circulating concentrations that reflect energy storage. While this afferent directive results in an appropriate efferent feeding behavior, it also modulates relevant neuroendocrine axes. We suggest that the glucocorticoid and GH axes be extended to include leptin feedback. Recent studies by Ahima and colleagues[50] also imply that leptin feedback be included in regulation of the thyroid and reproduction axes. During states of scarce fuel supply, leptin levels fall, yielding a signal for energy replenishment such as increased feeding and augmented glucocorticoid secretion. In addition, conservation of existent energy reserve for maintenance of basal physiological processes would prohibit energy used for growth and reproduction until the energy level is replenished; thus, these axes would be inhibited until the adequate fuel level is replenished as signaled by elevation of circulating leptin levels.

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