Mechanisms of Leptin Action and Leptin Resistance

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Annu. Rev. Physiol. 2008. 70:537–56

First published online as a Review in Advance on October 15, 2007

The *Annual Review of Physiology* is online at http://physiol.annualreviews.org

This article's doi: 10.1146/annurev.physiol.70.113006.100707

0066-4278/08/0315-0537$20.00

Key Words

hypothalamus, VTA, obesity, diabetes

Abstract

The adipose tissue–derived hormone leptin acts via its receptor (LRb) in the brain to regulate energy balance and neuroendocrine function. LRb signaling via STAT3 and a number of other pathways is required for the totality of leptin action. The failure of elevated leptin levels to suppress feeding and mediate weight loss in common forms of obesity defines a state of so-called leptin resistance. A number of mechanisms, including the leptin-stimulated phosphorylation of Tyr_{985} on LRb and the suppressor of cytokine signaling 3, attenuate leptin signaling and promote a cellular leptin resistance in obesity. Several unique features of the arcuate nucleus of the hypothalamus may contribute to the severity of cellular leptin resistance in this region. Other mechanisms that govern feeding behavior and food reward may also underlie the inception of obesity.

LEPTIN

The adipose tissue–derived hormone leptin is produced in proportion to fat stores. Circulating leptin serves to communicate the state of body energy repletion to the central nervous system (CNS) in order to suppress food intake and permit energy expenditure (1–3). Many of the physiological adaptations triggered by prolonged fasting can be attenuated by exogenously administered leptin, which falsely signals to the brain that energy stores are replete (3–5). Adequate leptin levels permit energy expenditure on the processes of reproduction, tissue remodeling, and growth and similarly regulate the autonomic nervous system, other elements of the endocrine system, and the immune system (3–5). Conversely, lack of leptin signaling due to mutation of leptin (e.g., *ob/ob* mice) or the leptin receptor (LR) (e.g., *db/db* mice) in rodents and humans results in increased food intake in combination with reduced energy expenditure and a phenotype reminiscent of the neuroendocrine starvation response (including hypothyroidism, decreased growth, infertility, and decreased immune function) in spite of obesity (1, 2, 6, 7).

LEPTIN RECEPTORS AND SITES OF LEPTIN ACTION

There are multiple LR isoforms, all of which are products of a single *Lepr* gene (8, 9). The *Lepr* gene contains 17 common exons and several alternatively spliced 3′ exons. In mice, the six distinct LR isoforms that have been identified are designated LRa–LRf. In all species, LR isoforms are divisible into three classes: secreted, short, and long. The secreted forms are either products of alternatively spliced mRNA species (e.g., murine LRe, which contains only the first 14 exons of *Lepr*) or proteolytic cleavage products of membrane-bound forms of LR. These secreted forms contain only extracellular domains that bind circulating leptin, perhaps regulating the concentration of free leptin (10).

Short-form LRs (LRa, LRc, LRd, and LRf in mice) and the long-form LR (LRb in mice) contain exons 1–17 of *Lepr* and therefore have identical extracellular and transmembrane domains as well as the same first 29 intracellular amino acids but diverge in sequence thereafter owing to the alternative splicing of 3′ exons. Short-form LRs contain exons 1–17 and terminate 3–11 amino acids after the splice junction for total intracellular domain lengths of 32–40 amino acids. LRc-, LRd-, and LRf-specific sequences are not well conserved among species. However, LRa (the most abundantly expressed isoform) is reasonably well conserved, as is LRb, which has an intracellular domain of approximately 300 residues (8, 9).

LRb is crucial for leptin action. Indeed, the originally described *db/db* mice lack LRb (but not other LR forms) as a consequence of a mutation that causes missplicing of the LRb mRNA; these mice closely resemble db^{3J}/db^{3J} mice (which are deficient in all LR isoforms) and leptin-deficient *ob/ob* animals (3). The function of short-form LRs is less clear, although proposed roles include the transport of leptin across the blood-brain barrier (BBB) and the production of circulating LR extracellular domain to complex with leptin (10, 11).

Many of the effects of leptin result from actions in the CNS, particularly in the hypothalamus, a site of high LRb mRNA expression (12–15). In the hypothalamus, leptin acts on neurons that directly or indirectly regulate levels of circulating hormones (e.g., thyroid hormone, sex steroids, and growth hormone) (12, 16, 17). Leptin action on these hypothalamic neurons also regulates the activity of the autonomic nervous system, although direct effects of leptin on LRb-containing neurons in the brainstem and elsewhere probably also have an important role (18). The effects of leptin on the immune system and vasculature appear to result from direct action on hematogenous cells that contain LRb (5, 19). Leptin may also regulate glucose homeostasis independently of its effects on adiposity; leptin regulates glycemia at least partly via the CNS,

but it may also directly regulate pancreatic β-cells and insulin-sensitive tissues (20–24).

LEPTIN REGULATION OF NEURAL NETWORKS AND NEUROPHYSIOLOGY

LRb is present in several tissues; the highest levels are in neurons of several nuclei of the hypothalamus, including the arcuate (ARC), dorsomedial (DMH), ventromedial (VMH), lateral hypothalamic area (LHA), and ventral premammillary (PMv) nuclei (12–14, 25). Other sites within the brain that have been shown to express functional LRb include the ventral tegmental area (VTA), brainstem [including the nucleus of the solitary tract (NTS) and dorsal motor nucleus of the vagus], and the periaqueductal gray matter, among others.

LRb action on two populations of ARC neurons is particularly well characterized. One population synthesizes neuropeptide Y (NPY) and agouti-related peptide (AgRP), and the other synthesizes pro-opiomelanocortin (POMC) (12, 15). POMC is processed to produce α-melanocyte-stimulating hormone (αMSH) in LRb/POMC neurons; αMSH signals anorexia (decreased appetite) by activating the melanocortin-4 receptor (MC4R) and the melanocortin-3 receptor (MC3R) (26–31). LRb stimulates the synthesis of POMC, activates LRb/POMC neurons (15, 32), and stimulates αMSH secretion (33). NPY is an orexigenic (appetite-stimulating) hormone that also suppresses the central LRb-mediated growth and reproductive axes (34–37). AgRP is an antagonist of αMSH/MC4R signaling as well as an inhibitor (inverse agonist) of endogenous MC4R activity (36, 38). Leptin acts via LRb to inhibit NPY/AgRP neurons and suppress the expression and secretion of NPY and AgRP (15, 32, 33). Thus, LRb signaling stimulates the production and secretion of anorectic neuropeptides and reciprocally suppresses levels of orexigenic peptides. Conversely, a decrease or deficiency in leptin action (e.g., during starvation or in *ob/ob* and *db/db* mice) stimulates appetite by the suppression of the synthesis of anorectic neuropeptides (e.g., POMC) and increased expression of orexigenic peptides (e.g., NPY and AgRP).

Although we now know a great deal about the mechanisms by which the ARC NPY/AgRP and POMC neurons function, numerous questions remain regarding the contributions of each circuit to the regulation of feeding in general and in response to leptin under physiological conditions. Although ablation of AgRP neurons results in hypophagia and ablation of POMC or central melanocortin receptors results in severe obesity (27, 39), deletion of LRb from POMC neurons or the restoration of LRb in the ARC of *db/db* animals results in only modest alterations in body weight (although these manipulations robustly modulate glucose homeostasis) (40, 41).

Furthermore, although interference with LRb → STAT3 (signal transducer and activator of transcription 3) signaling results in dramatic hyperphagia and obesity, deletion of STAT3 in ARC neurons only modestly impacts body energy homeostasis (42–44). Thus, although melanocortins and ARC neurons generally effect powerful appetitive signals, they may not constitute the majority of the leptin-mediated anorectic signal; the aggregate leptin signal is likely mediated in concert with many other populations of LRb-expressing neurons that require further analysis. Indeed, ARC LRb neurons comprise only 15–20% of the total number of LRb-expressing neurons within the CNS (25), and other populations of LRb neurons, including those in the VMH and VTA, clearly mediate important components of leptin action (45–47).

LEPTIN RECEPTOR SIGNALING

LRb belongs to the interleukin (IL)-6 receptor family of class 1 cytokine receptors, which contain an extracellular ligand-binding domain, a single transmembrane domain, and a cytoplasmic signaling domain (8, 48). Like

other cytokine receptors, LRb does not contain intrinsic enzymatic activity but instead signals via a noncovalently associated tyrosine kinase of the Jak kinase family (Jak2 in the case of LRb) (49–51). Leptin binding alters the conformation of the preformed LRb homodimer, enabling transphosphorylation and activation of the intracellular LRb-associated Jak2 (8, 52, 53). The activated Jak2 molecule then phosphorylates other tyrosine residues within the LRb/Jak2 complex to mediate downstream signaling (54, 55).

Signaling by cytokine receptors requires a proline-rich Box 1 motif critical for Jak kinase interaction and activation; additional, less-conserved sequences COOH-terminal to Box 1 (sometimes referred to as Box 2) are also important for Jak interactions and likely function in Jak isoform selectivity (48, 49, 51, 56). In the case of LRb, intracellular residues 31–36 (i.e., those immediately downstream of the alternative splice junction following amino acid 29) compose Box 2 and dictate Jak2 selectivity (51, 56). This Box 2 sequence is absent from all described short LR isoforms—consistent with the inability of these molecules to mediate leptin action in *db/db* animals (8, 51, 54).

Tyrosine kinase–dependent signaling generally proceeds via the phosphotyrosine-mediated recruitment of signaling proteins that contain specialized phosphotyrosine-binding domains (e.g., SH2 domains) (57). Each SH2 domain isoform recognizes phosphotyrosine in a specific amino acid context. Thus, although tyrosine phosphorylation acts as a molecular switch to recruit SH2-containing proteins, each tyrosine phosphorylation site recruits only specific SH2 isoforms because each isoform recognizes specific surrounding amino acids as well as the phosphotyrosine residue. For instance, the SH2 domain of the latent transcription factor STAT3 binds to phosphotyrosine in the context of a Y(P)XXQ motif (58, 59).

Understanding signaling by the LRb/Jak2 complex thus requires defining the tyrosine phosphorylation sites on LRb and Jak2 and the SH2 proteins that they recruit. There are three conserved residues on the intracellular domain of LRb: Tyr_{985}, Tyr_{1077}, and Tyr_{1138}. Data from our and other labs suggest that all three of these sites are phosphorylated and contribute to downstream leptin signaling (8, 54, 55, 60, 60a).

There are thus four tyrosine phosphorylation signaling pathways that can derive from LRb (**Figure 1**): those originating directly from Jak2 tyrosine phosphorylation sites and those emanating from the phosphorylation of Tyr_{985}, Tyr_{1077}, and Tyr_{1138} of LRb. The phosphorylation of Tyr_{985} creates a binding site for the COOH-terminal SH2 domain of the tyrosine phosphatase SHP-2, leading to the activation of the canonical p21ras → ERK signaling pathway in cultured cells (51, 55, 61).

Phosphorylation of Tyr_{1138} recruits STAT3 to the LRb/Jak2 complex, resulting in the tyrosine phosphorylation and subsequent nuclear translocation of STAT3 to mediate transcriptional regulation (54, 55). Among the STAT3-regulated genes is the SH2 domain–containing feedback inhibitor SOCS3 (suppressor of cytokine signaling 3) (55, 62). Following its STAT3-dependent production during leptin stimulation, SOCS3 binds to Tyr_{985} of LRb to mediate the inhibition of LRb → STAT3 signaling (63); SOCS3 also binds to a separate site on Jak2 (64, 65).

Tyr_{1077} mediates a crucial component of STAT5 (signal transducer and activator of transcription 5) phosphorylation and transcriptional regulation by leptin, although Tyr_{1138} also contributes to STAT5 activation (60, 60a). Tyr_{1077} does not regulate STAT3 signaling, although it may promote the increased phosphorylation of LRb Tyr_{985}.

Jak2 tyrosine phosphorylation during LRb stimulation may mediate some signals independently of tyrosine phosphorylation sites on LRb (55). The individual phosphorylation sites on Jak2 are beginning to be enumerated (66–73). Unfortunately, many more remain to be identified, and the binding partners and signals mediated by many sites are

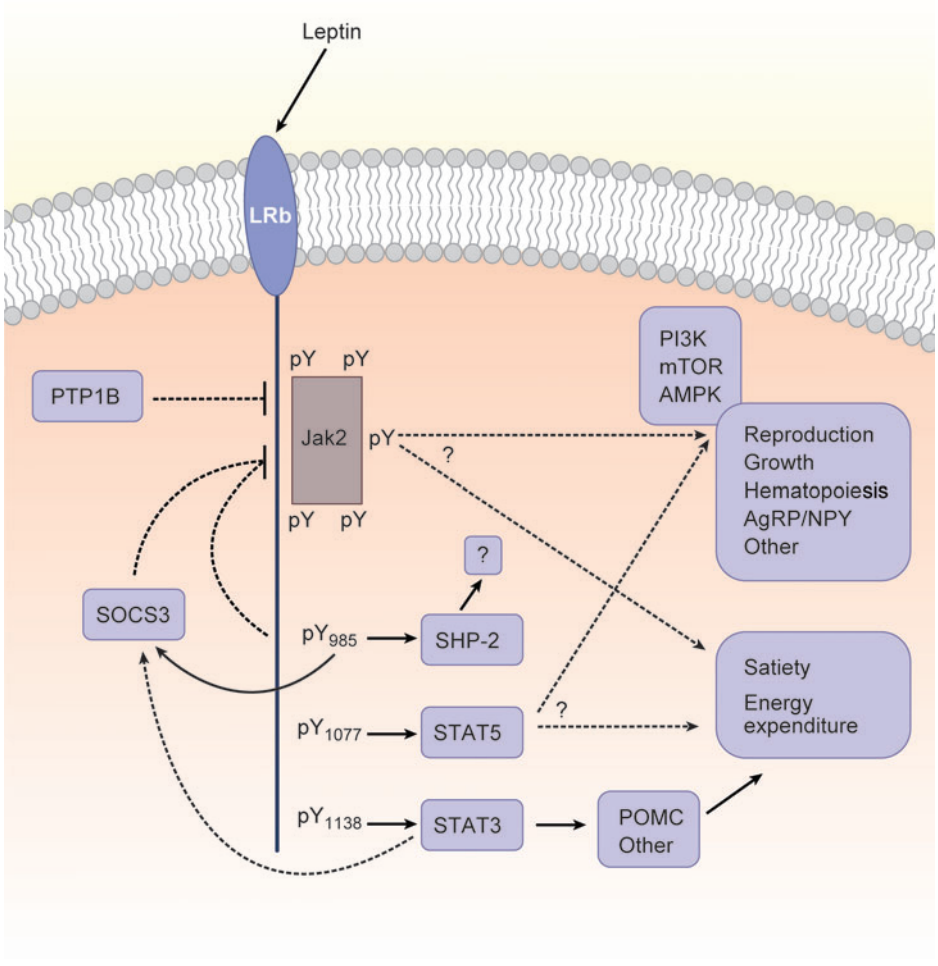

Figure 1

LRb signaling, feedback inhibition, and the regulation of physiology. Leptin binding to the extracellular domain of LRb, the functional leptin receptor isoform, mediates the activation of the intracellular, LRb-associated Jak2 tyrosine kinase, resulting in Jak2 autophosphorylation on tyrosine residues (pY) as well as the phosphorylation of three tyrosine residues on the intracellular tail of LRb: Y_{985}, Y_{1077}, and Y_{1138}. pY_{1138} recruits signal transducer and activator of transcription (STAT) 3, which is activated to mediate transcriptional events, including the transcription of pro-opiomelanocortin (POMC) and the inhibitory suppressor of cytokine signaling 3 (SOCS3) protein. pY_{1077} recruits and mediates the transcriptional activation of STAT5. pY_{985} recruits the tyrosine phosphatase SHP-2 and also binds to SOCS3 and mediates feedback inhibition of LRb signaling (*dotted lines*). The tyrosine phosphatase PTP1B, although not regulated by leptin in this manner, also inhibits LRb/Jak2 signaling. The cellular mechanisms by which LRb couples to the regulation of phosphatidylinositol 3-kinase (PI3K), mammalian target of rapamycin (mTOR), and AMP-activated protein kinase (AMPK) pathways remain unclear. Y_{1138}-mediated STAT3 signaling by LRb (presumably via POMC and additional mechanisms) is crucial to the regulation of anorexia and energy expenditure by leptin. Although Y_{985} clearly functions to attenuate LRb signaling in vivo, a role for Y_{985} and SHP-2 in promoting leptin action has not been defined. Leptin mediates permissive effects upon reproduction, growth, hematopoietic effects (e.g., immune and platelet function), and the inhibition of agouti-related protein (AgRP)/neuropeptide Y (NPY) neurons of Y_{1138} and Y_{985}, perhaps via pY sites on Jak2 or via pY_{1077}.

not known, limiting our understanding of the mechanisms by which Jak2-dependent signals are mediated. LRb stimulation mediates the tyrosine phosphorylation of IRS proteins and regulates the PI 3′-kinase pathway (74–76) as well as the AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) pathways (77, 78), although the molecular mechanisms by which LRb regulates these pathways remain unclear.

LRb SIGNALING VIA STAT3 MEDIATES A SUBSET OF LEPTIN ACTIONS

Thus far, roles for two signals mediated by LRb tyrosine phosphorylation sites—the $Tyr_{1138} \rightarrow$ STAT3 pathway and the $Tyr_{985} \rightarrow$ SOCS3/SHP2 pathway—have been examined in leptin action in vivo (**Figure 2**). We have directly addressed the contribution of the LRb-STAT3 pathway to physiology

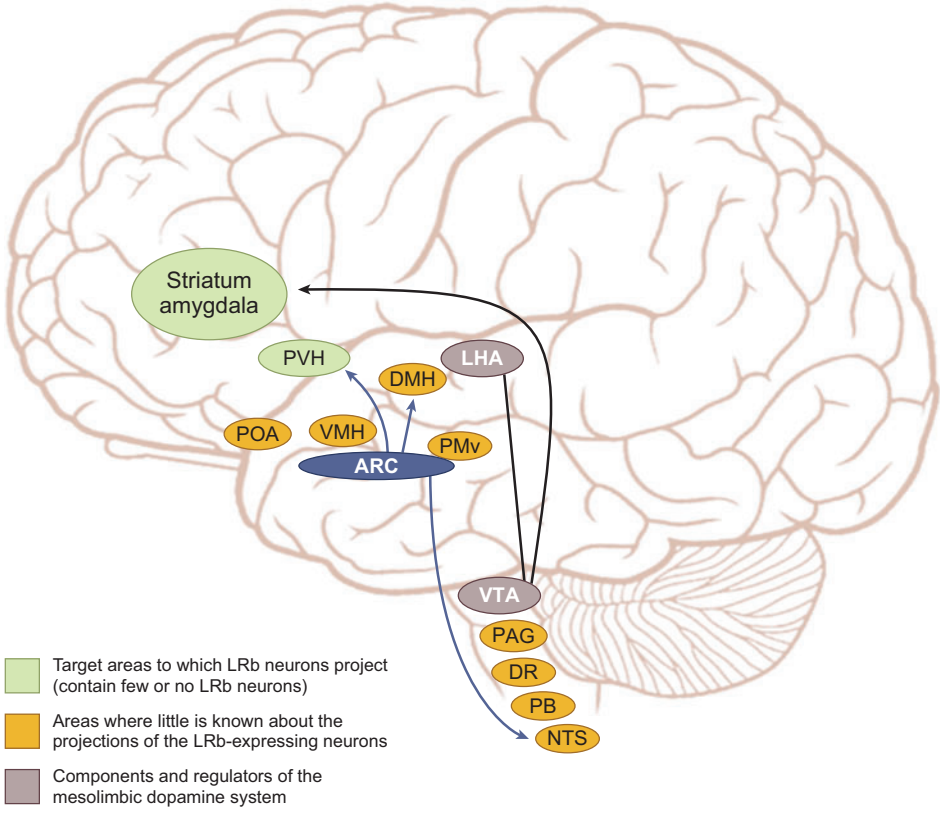

Figure 2

A distributed network of LRb-expressing neurons in the CNS regulates multiple neural processes. Shown in blue, yellow, and brown bubbles are brain regions containing significant populations of LRb-expressing neurons. Yellow bubbles indicate areas where little is known about the projections of the LRb-expressing neurons. Bubbles with arrows have LRb neurons with somewhat defined projection patterns. Target areas to which LRb neurons project but that contain few or no LRb neurons are denoted as light green bubbles. Components and regulators of the mesolimbic dopamine system are shown in brown bubbles. ARC, arcuate nucleus; PVH, paraventricular hypothalamic nucleus; VMH, ventromedial hypothalamic nucleus; DMH, dorsomedial hypothalamic nucleus; LHA, lateral hypothalamic area; PMv, ventral premammilary nucleus; POA, preoptic area; VTA, ventral tegmental area; PAG, periaqueductal gray; DR, dorsal raphe; PB, parabrachial nucleus; NTS, nucleus of the solitary tract.

by studying homologously targeted knock-in mice in which LRb is replaced by a mutant molecule (LRb^{S1138}) that contains a substitution mutation of Tyr_{1138} (the STAT3 binding site) (42). Although LRb^{S1138} fails to mediate STAT3 activation during leptin signaling, this mutant regulates all other known LRb signaling pathways. The use of the knock-in approach ensures that the expression pattern and levels of LRb^{S1138} mirror those of wild-type LRb.

Similar to *db/db* animals, mice homozygous for LRb^{S1138} expression (*s/s*) display hyperphagia and decreased energy expenditure, resulting in profound obesity in the face of dramatically increased serum leptin levels. The high circulating leptin levels in *s/s* animals not only correlate with increased adipose mass in these mice but also indicate resistance to the energy homeostatic effects of leptin (42). Feeding is similarly high in *s/s* and *db/db* mice, and thyroid function and energy expenditure are similarly decreased in these two mouse strains (79).

Important differences exist between the phenotypes of *s/s* mice (missing only the LRb-STAT3 signal) and *db/db* mice (devoid of all leptin signals), however (42). Whereas *db/db* animals are floridly diabetic and infertile and demonstrate decreased linear growth, *s/s* mice demonstrate greatly improved glucose tolerance compared with *db/db* mice. The *s/s* mice also retain fertility and demonstrate increased linear growth as well as immune and vascular reactivity to leptin compared with wild-type animals (42, 79–83).

Analysis of hypothalamic neuropeptide expression reveals that, similar to *db/db* mice, *s/s* mice have decreased POMC mRNA levels in the hypothalamus (42). By contrast, whereas *db/db* animals display dramatic induction of hypothalamic NPY mRNA, levels of NPY message are near normal in *s/s* animals. Furthermore, the activity of these AgRP/NPY neurons is appropriately suppressed in *s/s*, but not *db/db*, animals (84). These data suggest that LRb-STAT3 signaling is a crucial regulator of hypothalamic melanocortin action and that dysregulated melanocortin signaling (as opposed to alterations in NPY) may contribute to the obesity of *s/s* animals, although STAT3 presumably mediates other leptin effects in other LRb-expressing neurons. Hence, non-STAT3 LRb signals are critical regulators of neural activity and NPY expression in the LRb/NPY neuron.

Clearly, pathways independent of LRb → STAT3 regulate glycemic control, the function of hematopoietic and vascular cells, reproduction, growth, and NPY/AgRP neurons in response to leptin. The phenotype of the *s/s* animals does not suggest the irrelevance of non-STAT3 pathways in other aspects of energy balance, however, and reveals only that STAT3 signaling is important for the regulation of energy homeostasis. Thus, signals independent of Tyr_{1138} → STAT3 may contribute to energy balance as well as to the myriad leptin effects that are preserved in *s/s* mice.

LRb Tyr_{985} ATTENUATES LEPTIN ACTION IN VIVO

To understand the contribution of LRb Tyr_{985} to leptin action and inhibition in vivo, we generated mice in which LRb was homologously replaced by a mutant containing a substitution of Tyr_{985} that abrogates phosphorylation of the site and blocks SHP-2/SOCS3 recruitment (55, 61, 63, 85). Mutation of Tyr_{985} in vivo results in reduced feeding and adiposity, decreased orexigenic ARC neuropeptide expression, and increased baseline STAT3 activation in female *l/l* mice—all in the face of low leptin levels. Coupled with the increased sensitivity of *l/l* animals to exogenous leptin, these observations suggest that mutation of Tyr_{985} blocks the activation of an inhibitory Tyr_{985}-dependent LRb signal, ultimately leading to increased leptin sensitivity in vivo. These results suggest an important role for Tyr_{985} in the attenuation of leptin action in vivo, consistent with results from cultured cells suggesting an important role for Tyr_{985} in the inhibition of LRb signaling (63, 86, 87).

Leptin resistance: the failure of high levels of leptin in obese individuals to suppress feeding and prevent or mitigate obesity

Because Tyr_{985} of LRb recruits both SHP-2 and SOCS3 (63, 87, 88), the failure of LRb^{L985} to recruit either of these proteins may theoretically underlie the lean, leptin-sensitive phenotype of *l/l* mice. Many data from cultured cells and animals support a primary role for SOCS3 in the inhibition of LRb signaling, however, suggesting that SOCS3 (rather than SHP-2) mediates Tyr_{985}-dependent inhibition of LRb (61–63, 83, 89–91).

The phenotype of *l/l* mice also suggests that SHP-2 may not be required for the regulation of growth or reproduction by leptin and does not mediate essential anorectic signals. This finding contrasts with the obesity and impaired neuroendocrine function in animals with deletion of SHP-2 in the forebrain (91), consistent with the notion that disruption of SHP-2 alters signaling by numerous factors other than leptin and in a wide variety of neuronal populations (92, 93). The loss of SHP-2 recruitment by leptin in *l/l* animals may result in a diminution of anorectic function that is obscured by the enhancement of overall LRb signaling owing to the concomitant loss of inhibitory signals, however. Collectively, these findings suggest that LRb Tyr_{1138}- and Tyr_{985}-independent signals likely contribute to the regulation of growth, reproduction, and glucose homeostasis by leptin (42). These signals may include the LRb Tyr_{1077}/STAT5 pathway or signals mediated by the LRb-associated Jak2 independently of LRb tyrosine phosphorylation (3, 60, 74). Additionally, some possible downstream pathways include the PI 3′-kinase, mTOR, and AMPK pathways, although we cannot rule out the possibility that other uncharacterized signals may also participate.

LEPTIN RESISTANCE IN OBESITY

An absolute deficit of leptin does not underlie most cases of obesity: Indeed, most obese individuals exhibit elevated circulating leptin levels commensurate with their adipose mass (94–96). The apparent conundrum that this observation implies (why do elevated leptin levels not act to decrease feeding and thus prevent obesity?) has given rise to the notion of the existence of physiological leptin resistance. Simply put, the failure of high levels of leptin to suppress feeding and decrease body weight/adiposity to prevent or mitigate obesity suggests a relative resistance to the catabolic effects of leptin action in obesity.

A number of mechanisms have been proposed to explain leptin resistance; these include alterations in the transport of leptin across the BBB, alterations in cellular LRb signaling, perturbations in developmental programming, and others (97–99; 101). Indeed, each of these mechanisms may contribute to the totality of leptin resistance. Although the absolute lack or genetic alteration of LRb does not underlie most leptin resistance (95, 100), the preponderance of data confirm that alterations in cellular LRb signaling, especially in the ARC, play a crucial role in leptin resistance (98, 101, 102).

LRb SIGNAL ATTENUATION AND EVIDENCE FOR CELLULAR LEPTIN RESISTANCE IN OBESITY

The concept of leptin resistance is analogous to the syndrome of insulin resistance, in which elevated levels of insulin are required to mediate adequate glucose disposal and metabolic control. In the case of insulin resistance, a number of intracellular pathways contribute to the attenuation of insulin signaling in insulin-responsive tissues such as muscle and liver (103). Indeed, diet-induced obese (DIO) animals (in which consumption of a palatable, calorically dense diet promotes obesity) are leptin resistant, displaying decreased anorectic response and decreased amplitude of maximal LRb signaling in the hypothalamus in response to high-dose leptin treatment, as evidenced by decreased STAT3 phosphorylation and neuropeptide release compared with controls (33, 98, 101, 102). Furthermore, maximal

leptin-stimulated neuropeptide release is impaired in explanted tissues from DIO mice, demonstrating the preservation of impaired leptin signaling outside of the physiological milieu of DIO mice (33).

Others and we have therefore undertaken to define the cellular mechanisms that contribute to the attenuation of LRb signaling, with the idea that these mechanisms may contribute to a cellular leptin resistance similar to the insulin signaling defects in insulin resistance (98). As noted above, SOCS3 binds to LRb Tyr_{985} and Jak2 to impair LRb signaling in cultured cells (63). Additionally, in mice, decreasing SOCS3 expression in the whole body or deleting SOCS3 in neurons increases the amplitude of LRb signaling, resulting in animals that are leaner than wild types at baseline and that are resistant to DIO (90, 104). As detailed above, LRb Tyr_{985} also mediates the attenuation of LRb signaling in cultured cells, and mutation of this residue in *l/l* mice results in augmented leptin sensitivity, leanness, and resistance to DIO (63, 85, 87, 88). Thus, Tyr_{985} and SOCS3 attenuate LRb signaling and contribute to leptin resistance.

The tyrosine phosphatase PTP1B dephosphorylates Jak2 to diminish LRb signaling in cultured cells, and whole-body or neuron-specific deletion of PTP1B increases leanness and leptin sensitivity (105–107). Neural PTP1B expression or activity is not altered by leptin or adiposity, however, suggesting that, although PTP1B physiologically attenuates leptin action and thus may represent an important therapeutic target, it may not underlie altered leptin signaling in obesity. Indeed, although neuronal deletion of PTP1B renders animals lean and leptin sensitive, the effect of PTP1B on adiposity is independent of DIO (that is, the increased leanness of neuronal PTP1B knockout mice relative to controls does not differ by diet) (105).

Leptin (which is increased in obesity) itself stimulates the phosphorylation of LRb Tyr_{985} to limit LRb signaling (63, 85, 87, 88), and SOCS3 expression increases in response to leptin and is elevated in the hypothalami of obese animals (62, 102, 108, 109). In addition to leptin, other cytokines promote SOCS3 accumulation. Thus, increased activity in any of these metabolic and inflammatory pathways has the potential to impair LRb signaling, and the convergence of all these signaling systems upon SOCS3 mirrors some of the phenotypes that comprise the metabolic syndrome. Thus, Tyr_{985} and SOCS3 contribute to cellular leptin resistance, specifically in states of obesity, and leptin/obesity activate these feedback signals to attenuate LRb signaling at high leptin levels, as found in obesity. This is not to say that increased leptin and/or obesity block LRb signaling to such an extent that LRb activity at these elevated circulating levels falls below that observed in lean controls with lower leptin levels, however. Rather, each increase in circulating leptin levels yields a smaller and smaller increase in LRb signaling over the baseline observed at low leptin levels (**Figure 3**). Indeed, DIO mice with several-fold increases in circulating leptin levels demonstrate only slightly increased baseline LRb signaling compared with normal, chow-fed mice (but this would presumably support some continued increase in Tyr_{985} phosphorylation and SOCS3 expression) (85). Thus, baseline LRb signaling in DIO mice, although modestly increased, is not proportional to their degree of hyperleptinemia. This LRb signal attenuation is also evident by the substantially reduced response to acute high-dose leptin administration (33, 85, 101).

THE ARCUATE NUCLEUS AS A CRUCIAL SITE OF CELLULAR LEPTIN RESISTANCE

The cellular leptin resistance phenotype of DIO animals is most prominently detected in the ARC relative to other hypothalamic sites (33, 102). Furthermore, the increased expression of SOCS3 in seasonally obese rodents is localized to the ARC (108, 109). This ARC specificity of cellular leptin resistance and increased SOCS3 expression raises the

question of how the ARC differs from other hypothalamic sites. Potentially increased access of leptin and other factors from the circulation into the ARC relative to other hypothalamic sites (where leptin access is limited by transport mechanisms across the BBB) may represent one such mechanism. Indeed, this notion finds support in our recent data demonstrating that endogenous circulating leptin (in untreated, ad libitum–fed mice) promotes increased LRb signaling in ARC neurons compared with LRb neurons in other sites (109a). Indeed, the time course of LRb signaling is delayed in non-ARC neurons relative to ARC neurons in response to peripheral leptin administration but is similar between hypothalamic sites after central leptin administration (which circumvents the BBB) (109a). This result is consistent with differential access of the ARC LRb neurons to circulating leptin (as opposed to intrinsic differences in the leptin responsiveness of the LRb neurons among sites).

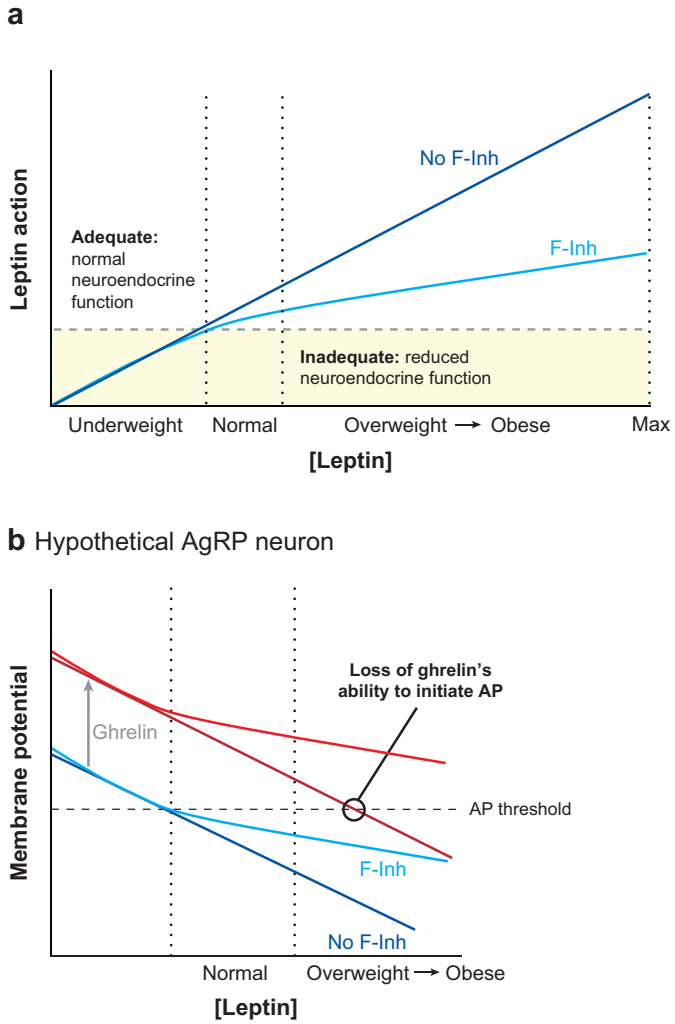

Figure 3

Theoretical functional consequences of the attenuation of leptin action in obesity. (*a*) Shown is a theoretical graph of how leptin action on body weight (e.g., via STAT3 phosphorylation) and on the reproductive axis (via an unknown signaling pathway) varies with leptin concentration in the presence or absence of feedback inhibition (F-Inh) mechanisms that attenuate leptin action in proportion to leptin levels and/or adiposity. At low leptin levels, at which the effect of F-Inh is minimal, the curves for F-Inh and no F-Inh overlie. The lines diverge as leptin and F-Inh increase. With increasing concentrations of endogenous circulating leptin in the case of F-Inh, leptin action increases modestly. The amplitude of the leptin signal in response to a single large dose of leptin (seen at max) is attenuated in animals with increased baseline leptin levels (at which F-Inh levels are high before the leptin dose is given) compared with animals with low baseline levels of leptin and low F-Inh; the latter group of animals should demonstrate a response analogous to the maximum of the no F-Inh line. (*b*) Mechanism by which the presence of F-Inh enables the detection of energy flux via hormones such as ghrelin in the face of high leptin levels. Taken is the hypothetical case for a leptin-inhibited, ghrelin-activated agouti-related protein (AgRP) neuron. Curves for the effect of leptin on the membrane potential are shown for various leptin levels for the cases of no F-Inh (*dark blue*) and F-Inh (*blue*). Also shown is the effect for this theoretical neuron of a high physiological dose of ghrelin, which relatively depolarizes the neuron (*gray arrow*) and shifts the leptin curves (*red*) as shown. When there is no F-Inh, ghrelin is incapable of depolarizing the theoretical neuron to the point of action potential (AP) generation (AP threshold) at high levels of leptin, whereas the attenuation of leptin action by F-Inh mechanisms at chronically high leptin levels permits the detection of ghrelin/energy flux even in the face of high leptin levels.

Furthermore, peripheral application of the BBB-impermeant retrograde neuronal tracer fluorogold reveals a substantial population of highly leptin-sensitive LRb neurons that directly contact the circulation in the ARC, but not elsewhere in the hypothalamus (109a). Hence, a population of ARC LRb neurons is directly exposed to circulating leptin levels and poised to respond more sensitively to circulating leptin (and other factors) compared with LRb neurons at other sites. These ARC LRb neurons may be more prone to the development of cellular leptin resistance than other LRb neurons owing to their increased exposure to high leptin levels or to other potential circulating mediators of cellular leptin resistance in obesity.

WHEREFORE CELLULAR LEPTIN RESISTANCE?

We are faced with the challenge of explaining the need for and the physiological consequences of feedback mechanisms that limit LRb signaling in hyperleptinemic/obese states. For some organisms, such as seasonal mammals, there is a periodic need to increase energy stores, and thus these feedback mechanisms may work in concert with other processes that increase energy intake to promote seasonal energy storage. For nonseasonal animals, such as humans, another potential explanation for feedback inhibition of LRb signaling arises from the need to sense not only the content of body energy stores but also the flux of energy, as detailed for the case of reproduction in *On Fertile Ground: A Natural History of Reproduction* (109b). Even when energy stores are relatively high (resulting in high circulating leptin levels), it is important to evaluate the rate of energy expenditure (energy flux) to determine if the organism is in a positive or a negative energy balance and thus enable the organism to further increase or maintain food consumption despite already elevated energy stores. Specific instances in which expenditure may be high and caloric intake must be increased (even in the face of normal energy stores) include pregnancy, lactation, and intensive exercise. Indicators of high energy flux that must be sensed even if circulating levels indicate significant energy stores include falls in leptin levels (even within the high end of the normal physiological range) as well as opposing and short-term acting factors like the gut hormone ghrelin. However, in a system in which increases in circulating leptin levels linearly amplify LRb signaling (leptin rises and falls in direct proportion to energy stores at all levels of adiposity), it is difficult to detect alterations in energy flux at high leptin levels because very elevated LRb signaling could overwhelm opposing signals like ghrelin (**Figure 3**). In contrast, the presence of a leptin-stimulated feedback mechanism prevents unlimited leptin action during hyperleptinemia. Thus, this system protects the ability to detect alterations in energy flux by ensuring that signals like ghrelin are not overwhelmed at relatively high leptin levels if negative energy balance exists.

In addition to mediators of cellular leptin resistance, such as Tyr_{985} and SOCS3, other mechanisms of cellular leptin resistance and any mechanism of leptin resistance that is increased by adiposity or leptin levels (including alterations in BBB leptin transport) should act in this manner.

OTHER POTENTIAL MECHANISMS OF LEPTIN RESISTANCE

Although cellular leptin resistance is physiologically relevant and even desirable to permit the detection of energy flux in states in which increased adipose stores exist, such mechanisms that require leptin or increased adiposity to initiate leptin resistance cannot underlie the inception of obesity but can only contribute to its stability. For example, mice that are put on a high-fat diet to induce DIO begin with a perfectly acting LRb signaling system. The diet, rather than alteration of the LRb system itself, must trigger the increased energy intake, although the developing

feedback inhibition/cellular leptin resistance exacerbate and stabilize the ensuing increase in body weight. Although genetic variability in factors that attenuate LRb signaling (e.g., PTP1B, SOCS3) may underlie a cellular leptin resistance that causes obesity, there is clearly a strong environmental component to obesity, as evidenced by the rapidly increasing rates of obesity in industrialized countries today. Some evidence exists for developmental alterations in neural and other systems that may underlie some propensity to obesity, but the ready availability of palatable, calorically dense food (the basis for DIO in experimental animals) clearly plays a dominant role. Indeed, the obesity and cellular leptin resistance of DIO animals are reversed by replacing the palatable calorie-dense chow used to promote obesity with standard chow (33).

Although some of the obesogenic effects of tasty foods may be due to their nutrient content, the hedonic or rewarding properties of these foods also contribute (110, 111). Leptin regulates the perception of the rewarding value of palatable food (as well as that of other addictive substances, such as drugs of abuse) (112–115).

Leptin regulates a broadly distributed network of LRb-expressing neurons in the brain to orchestrate an array of neural processes (**Figure 2**). Some of the neural mechanisms by which leptin may control food reward are beginning to be elucidated via the investigation of the interaction of leptin with the mesolimbic dopamine (DA) system. The core of the mesolimbic DA system lies in a set of DA neurons in the ventral tegmental area that project forward to innervate the striatum (nucleus accumbens, caudate/putamen), amygdala, and prefrontal cortex (111). It is by acting upon this system that drugs of abuse generally exert their reinforcing effects, and the activity of this system is clearly important to mediate the incentive salience of food and other natural rewards. Although ARC LRb neurons do not project to the VTA and there is little evidence for the modulation of the mesolimbic circuitry by NPY or melanocortin action, a number of research groups have demonstrated the presence of LRb-expressing VTA DA neurons and proven the ability of leptin to alter the physiology of this system (45, 47, 116, 117). Additionally, feeding and leptin modulate the reward associated with intracranial self-stimulation specifically in the LHA, which is mediated by the mesolimbic reward circuitry (116, 118). Indeed, we have identified a novel population of LRb-expressing neurons in the LHA that project to the VTA to regulate the mesolimbic DA system. Thus, leptin acts via multiple ARC-independent systems to control the VTA and the mesolimbic DA system at its inception in the VTA, and these sites of leptin action likely regulate the incentive salience of food.

How then is the action of leptin to regulate the perception of food reward overwhelmed to promote obesity in the face of plentiful tasty food? Leptin is only one of many inputs into the mesolimbic DA system and other neural pathways that regulate the perception of food reward, and physiological leptin levels may not be able to suppress the myriad other signals that compel us to consume tasty food. Although leptin may reasonably inhibit the drive to overeat foods with only modestly rewarding properties, leptin may be insufficient to effectively compete with the rewarding properties of more palatable treats because these more-rewarding foods engage powerful neural responses that oppose leptin within the mesolimbic DA system and elsewhere.

Indeed, endogenous (and exogenous) cannabinoids modulate the mesolimbic DA system and exert powerful anorectic signals. The finding that inhibitors of endocannabinoid action promote weight loss speaks to the importance of this system in energy balance (119). Furthermore, although leptin regulates the production of endogenous cannabinoids to some extent, many other factors, including stress, also contribute to their regulation.

Where does this leave us in terms of leptin resistance, cellular leptin resistance, the problem of plentiful calorie-dense foods, and therapeutic alternatives? First, many lines of

evidence suggest that, even if cellular leptin resistance or other obesity-induced mechanisms of leptin resistance may not be the initiating insult in obesity, it clearly contributes to the ability to become and remain obese, and the blockade of processes that mediate leptin signal attenuation remains an attractive potential therapeutic modality. Furthermore, the investigation of the leptin signaling system has led us to a more detailed and general understanding of the regulatory mechanisms of food intake, including the melanocortin or the mesolimbic VTA/DA system, that can be considered general tools in the regulation of feeding that are employed by several peptides (e.g., leptin, ghrelin, serotonin, neurotensin, etc.). A more detailed understanding of the widely distributed network of LRb neurons in several poorly investigated CNS sites and the neural mechanisms by which leptin and other cues (nutritional, taste, etc.) regulate the perception of food reward will likely reveal additional potential therapeutic targets.

DISCLOSURE STATEMENT

M.G.M. and H.M. are not aware of any biases that might be perceived as affecting the objectivity of this review.

M.A.C. is Chief Scientific Officer of, and owns stock in, Orexigen Therapeutics, Inc., a company that is developing pharmaceutical approaches to treat obesity and is developing combination therapies that attempt to bypass leptin resistance. The work described in this manuscript was not supported by Orexigen Therapeutics. Oregon Health and Sciences University (OHSU) and M.A.C. have a significant financial interest in Orexigen Therapeutics, which may have a commercial interest in the results of this research and technology; this potential conflict has been reviewed and managed by the OHSU Conflict of Interest in Research Committee and the Integrity Program Oversight Council.

In the past M.A.C. has received grant support from the NIH (RR 0163, DK 62202), Oregon National Primate Research Center, and Orexigen Therapeutics. In the past M.A.C. has received compensation from Orexigen Therapeutics, Novo Nordisk, Merck & Co., Semaphore Pharmaceuticals, Konovo Inc., 7TM Pharma, Ipsen, and Amylin Pharmaceuticals.

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