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REVIEW

LRb signals act within a distributed network of leptin-responsive neurones to mediate leptin action

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Abstract

The adipose tissue-derived hormone, leptin, acts via its receptor (LRb) in the brain to regulate energy balance and neuroendocrine function. In order to understand leptin action we have explored the physiological function of LRb signalling pathways, defining important roles for signal transducer and activator of transcription-3 (STAT3) in positive signalling and for LRbTyr$_{985}$-mediated feedback inhibition in leptin signal attenuation. As the cells on which leptin acts are not homogeneous, but rather represent a broadly distributed network of neurones with divergent projections and functions, it is also crucial to consider how each of these populations responds to LRb signals to contribute to leptin action. While well-known LRb-expressing neurones within the arcuate nucleus of the hypothalamus mediate crucial effects on satiety and energy expenditure, other populations of LRb-expressing neurones in the ventral tegmental area and elsewhere likely control the mesolimbic dopamine system. Additional populations of LRb-expressing neurones likely contribute to other aspects of neuroendocrine regulation. It will be important to define the molecular mechanisms by which leptin acts to regulate neurophysiology in each of these LRb-expressing neural populations in order to understand the totality of leptin action.
Keywords dopamine, energy balance, hypothalamus, leptin, STAT3.

Leptin

The adipose tissue-derived hormone leptin is produced in proportion to fat stores. Circulating leptin communicates the level of energy reserves in the periphery to the central nervous system in order to suppress food intake and permit energy expenditure (Friedman & Halaas 1998, Bates & Myers 2003, Elmquist *et al.* 2005, Morton *et al.* 2006). Adequate leptin levels permit energy expenditure on the processes of reproduction and growth, and similarly regulate other elements of the endocrine and immune systems (Ahima *et al.* 1996, Lord *et al.* 1998, Bates & Myers 2003). Conversely, lack of leptin signalling as a consequence of 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 thus obesity), plus neuroendocrine dysfunction (including hypothyroidism, decreased growth, infertility and decreased immune function) (Montague *et al.* 1997, Clement *et al.* 1998, Elmquist *et al.* 1998b, Friedman & Halaas 1998). Many of the effects of leptin are attributable to effects in the central nervous system (CNS), particularly in the hypothalamus, a site of high LRb mRNA expression (Elmquist *et al.* 2005, Morton *et al.* 2006).

Leptin receptors

A single *Lepr* gene directs the production of multiple alternatively-spliced LR isoforms that are divisible into three classes: secreted, short and long (Chua *et al.* 1997, Tartaglia 1997). The secreted forms are either products of alternatively spliced mRNA species (e.g. murine LRe,

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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 (Ge *et al.* 2002).

Short form LRs (including LRa in mice) and the single long form LR (LRb in mice) possess identical extracellular and transmembrane domains as well as the same first 29 intracellular amino acids, but diverge in sequence thereafter. Short form LRs terminate 3–11 amino acids after the splice junction for total intracellular domain lengths of 32–40 amino acids. LRb possesses an intracellular domain of approximately 300 residues and is the crucial receptor isoform for leptin action (Chua *et al.* 1997, Tartaglia 1997). Indeed, the originally described *db/db* mice lack LRb (but not other LR forms) as a consequence of a mutation that causes mis-splicing 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 (Bates & Myers 2003). The function of short-form LRs is less clear, although proposed roles include the transport of leptin across the blood-brain barrier and the production of circulating LR extracellular domain to complex with leptin.

Leptin receptor signalling

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 signalling domain (Taga & Kishimoto 1997, Tartaglia 1997). Like other cytokine receptors, LRb does not contain intrinsic enzymatic activity, but instead signals via a non-covalently associated tyrosine kinase of the Janus kinase (JAK) family – Jak2 in the case of LRb (Ihle & Kerr 1995, Taniguchi 1995, Kloek *et al.* 2002). Leptin binding alters the conformation of the preformed LRb homodimer, enabling transphosphorylation and activation of the intracellular LRb-associated Jak2 (Devos *et al.* 1997, Tartaglia 1997, Couturier & Jockers 2003). The activated Jak2 molecule then phosphorylates other tyrosine residues within the LRb/Jak2 complex to mediate downstream signalling (White *et al.* 1997, Banks *et al.* 2000, Hekerman *et al.* 2005).

Tyrosine kinase-dependent signalling generally proceeds via the phosphotyrosine-dependent recruitment of signalling proteins that contain specialized phosphotyrosine-binding domains [e.g. Src homology 2 (SH2) domains] (Koch *et al.* 1991). Each SH2 domain isoform recognizes phosphotyrosine in a specific amino acid context. Thus, while tyrosine phosphorylation acts as a molecular switch to recruit SH2-containing proteins, each tyrosine phosphorylation site recruits only specific SH2 isoforms, as SH2 domains recognize the 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 (Songyang *et al.* 1993, Haan *et al.* 1999). Understanding signalling 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 tyrosine residues on the intracellular domain of LRb – Tyr_{985}, Tyr_{1077} and Tyr_{1138} – and data from our laboratory and others suggest that all these sites are phosphorylated and contribute to downstream leptin signalling (Tartaglia 1997, White *et al.* 1997, Banks *et al.* 2000, Hekerman *et al.* 2005) (and our unpublished data).

There are thus four primary intracellular signalling pathways that emanate from LRb (Fig. 1) – those originating directly from Jak2 tyrosine phosphorylation sites plus those emanating from 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, SH2-containing tyrosine phosphatase-2, aka PTPN II (SHP-2), leading to the activation of the canonical p21ras → ERK signalling pathway in cultured cells (Banks *et al.* 2000, Bjorbaek *et al.* 2001, Kloek *et al.* 2002).

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 (White *et al.* 1997, Banks *et al.* 2000). Among other genes, STAT3 mediates the transcription of the SH2 domain-containing feedback inhibitor, suppressor of cytokine signalling (SOCS)-3 (Bjorbaek *et al.* 1998, Banks *et al.* 2000). SOCS3 binds to Tyr_{985} of LRb to mediate inhibition of LRb → STAT3 signalling (Bjorbaek *et al.* 2000); SOCS3 also binds to a separate site on Jak2 itself (Sasaki *et al.* 2000).

Tyr_{1077} mediates a crucial component of STAT5 phosphorylation and transcriptional regulation by leptin, although Tyr_{1138} also contributes to STAT5 activation (Hekerman *et al.* 2005, Gong *et al.* 2007). Tyr_{1077} does not regulate STAT3 signalling, although it may promote the increased phosphorylation of LRb Tyr_{985}.

Jak2 tyrosine phosphorylation during LRb stimulation mediates some signals independently of tyrosine phosphorylation sites on LRb, at least in cultured cells (Banks *et al.* 2000). The individual phosphorylation sites on Jak2 are beginning to be enumerated (Feng *et al.* 1997, Carpino *et al.* 2002, Argetsinger *et al.* 2004, Feener *et al.* 2004, Kurzer *et al.* 2004, Matsuda *et al.* 2004, Funakoshi-Tago *et al.* 2006, Ishida-Takahashi *et al.* 2006), but many more remain to be

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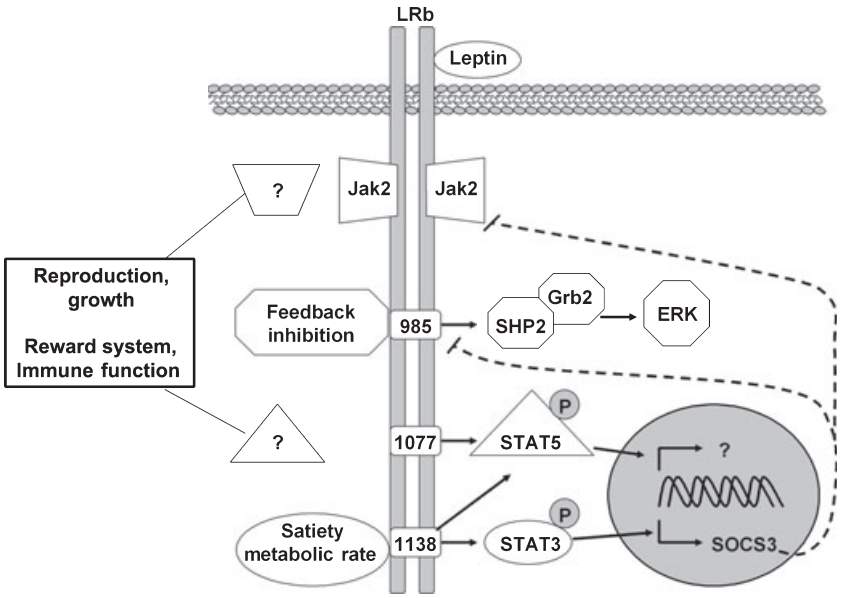

Figure 1 Linking LRb signalling to the regulation of mammalian physiology. Shown is a schematic of cellular LRb signalling and the roles of various signals in the regulation of physiology. Leptin binding to the extracellular domain of LRb activates the Jak2 tyrosine kinase that is constitutively associated with the intracellular domain of the receptor. This Jak2 activation results in the tyrosine phosphorylation of various sites on Jak2 and three sites on the receptor, each of which regulates different signalling pathways. LRb Tyr_{985} binds to SHP-2 to regulate the ERK pathway via GRB-2. Tyr_{1138} recruits STAT3 and contributes, along with Tyr_{1077}, to the activation of STAT5. STAT3 and STAT5 are each latent transcription factors that translocate to the nucleus during LRb signalling to mediate transcriptional events. STAT3 prominently activates the transcription of the inhibitory SOCS3, which attenuates LRb/Jak2 signalling by binding to Tyr_{985} and Jak2. Tyr_{1138} plays a prominent role in the regulation of satiety and metabolic rate *in vivo*, while Tyr_{985} primarily attenuates LRb signalling. Neither of these sites are required for reproduction or growth. The mechanisms by which LRb regulates other systems, including the mesolimbic dopamine/reward system and the immune system, also remain unclear.

identified. Unfortunately, the binding partners and signals mediated by many Jak2 phosphorylation sites remain unknown, limiting our understanding of the mechanisms by which Jak2-dependent signals are mediated. LRb stimulation regulates the phosphatidylinositol 3′-kinase (PI 3'-kinase), AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) pathways in the hypothalamus, and each of these hypothalamic pathways contributes to the regulation of feeding (Niswender *et al.* 2001, Minokoshi *et al.* 2004, Xu *et al.* 2005, Cota *et al.* 2006, Plum *et al.* 2006). The mechanisms by which LRb controls these pathways remain unclear, however.

LRb signalling via STAT3 mediates a subset of leptin actions

Thus far, roles for two signals mediated by LRb tyrosine phosphorylation sites have been examined in leptin action *in vivo*: the $Tyr_{1138} \rightarrow$ STAT3 pathway and the $Tyr_{985} \rightarrow$ SOCS3/SHP2 pathway (Fig. 1). We have directly addressed the contribution of the LRb-STAT3 pathway to physiology 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) (Bates *et al.* 2003). Although LRb^{S1138} fails to mediate activation of STAT3 during leptin signalling, this mutant regulates all other LRb signalling pathways normally. The use of the 'knock-in' approach ensures that the expression pattern and levels of LRb^{S1138} mirror that 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 (Bates *et al.* 2003). 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 (Bates *et al.* 2004).

However, 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) (Bates *et al.* 2003). Whereas *db/db* animals are floridly

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diabetic, infertile and demonstrate decreased linear growth, *s/s* mice exhibit greatly improved glucose tolerance compared with *db/db* mice, retain relatively normal gonad function and demonstrate increased linear growth compared with wild-type animals (Bates *et al.* 2003, 2004, 2005, Buettner *et al.* 2006). The cellularity of the thymus and thrombosis in response to vessel injury (both mediated by the effects of leptin on haematopoietic-derived cells) are also enhanced in *s/s* animals, though diminished in *db/db* mice (Bodary *et al.* 2002, 2007, Dunn *et al.* 2005).

Clearly, LRb → STAT3-independent pathways mediate a number of physiological responses to leptin. It is also important to note, however, that the phenotype of the *s/s* animals does not suggest the irrelevance of non-STAT3 pathways in other aspects of energy balance, only that STAT3 signalling is important for the regulation of energy homeostasis. Thus, Tyr_{1138} → STAT3-independent signals may contribute to energy balance as well as to the myriad other effects of leptin that are normal in *s/s* mice.

LRb Tyr_{985} attenuates leptin action *in vivo*

In order 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 LRb^{L985}, which contains a non-phosphorylatable substitution mutation of Tyr_{985} that blocks SHP2/SOCS3 recruitment (Banks *et al.* 2000, Bjorbaek *et al.* 2000, 2001, Bjornholm *et al.* 2007). Mutation of Tyr_{985} *in vivo* results in reduced feeding and adiposity, 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 that suggest an important role for Tyr_{985} in the inhibition of LRb signalling (Carpenter *et al.* 1998, Li & Friedman 1999, Bjorbaek *et al.* 2000).

As Tyr_{985} of LRb recruits both SHP-2 and SOCS3 (Carpenter *et al.* 1998, Li & Friedman 1999, Bjorbaek *et al.* 2000), the failure of LRb^{L985} to recruit either of these proteins could theoretically underlie the lean, leptin-sensitive phenotype of *l/l* mice. However, most data from cultured cells and animals support a primary role for SOCS3 in the inhibition of LRb signalling (Bjorbaek *et al.* 1998, 2000, 2001, Howard *et al.* 2004, Mori *et al.* 2004, Zhang *et al.* 2004, Dunn *et al.* 2005). SOCS3 mRNA expression was similar between +/+ and *l/l* mice, suggesting the effect of LRb Tyr_{985} in the attenuation of leptin action *in vivo* is not the result of differences in SOCS3 expression.

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 (Zhang *et al.* 2004), suggesting that disruption of SHP-2 alters signalling by numerous factors other than leptin, and in a wide variety of neuronal populations (Feng 1999, Keilhack *et al.* 2005). It is possible, however, that the loss of SHP-2 recruitment by leptin in *l/l* animals could result in a diminution of anorectic function that is obscured by the enhancement of overall LRb signalling due to the concomitant loss of inhibitory signals in LRb^{L985}. Overall, however, LRb Tyr_{1138}- and Tyr_{985}-independent signals probably contribute to the regulation of growth, reproduction, haematopoietic function and glucose homeostasis by leptin (Bates *et al.* 2003). The LRb Tyr_{1077}/STAT5 pathway, or signals mediated by the LRb-associated Jak2 independently of LRb tyrosine phosphorylation (Niswender *et al.* 2001, Bates & Myers 2003, Hekerman *et al.* 2005), are likely candidates to mediate the control of these Tyr_{985}/Tyr_{1138}-independent actions by leptin. Possible downstream pathways include the PI 3′-kinase, mTOR and AMPK pathways, although other uncharacterized signals could also participate.

Leptin regulation of neural networks and neurophysiology

LRb is present in several tissues, with the highest levels in neurones of several nuclei of the hypothalamus, including the arcuate (ARC), dorsomedial (DMH), ventromedial (VMH) and ventral pre-mammillary (PMv) nuclei and the lateral hypothalamic area (LHA) (Elmquist *et al.* 1998a, 2005, Baskin *et al.* 1999, Leshan *et al.* 2006, Morton *et al.* 2006) (Fig. 2). 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 the periaqueductal grey matter, among others.

LRb action in the ARC

Leptin action is particularly well characterized in two populations of ARC neurones, both of which express LRb. One population synthesizes neuropeptide Y (NPY) and agouti-related peptide (AgRP) and the other synthesizes pro-opiomelanocortin (POMC) (Elmquist *et al.* 2005, Morton *et al.* 2006). POMC is processed to produce α-melanocyte-stimulating-hormone (α-MSH) in LRb/POMC neurones, which signals anorexia (decreased appetite) by activating the melanocortin-4

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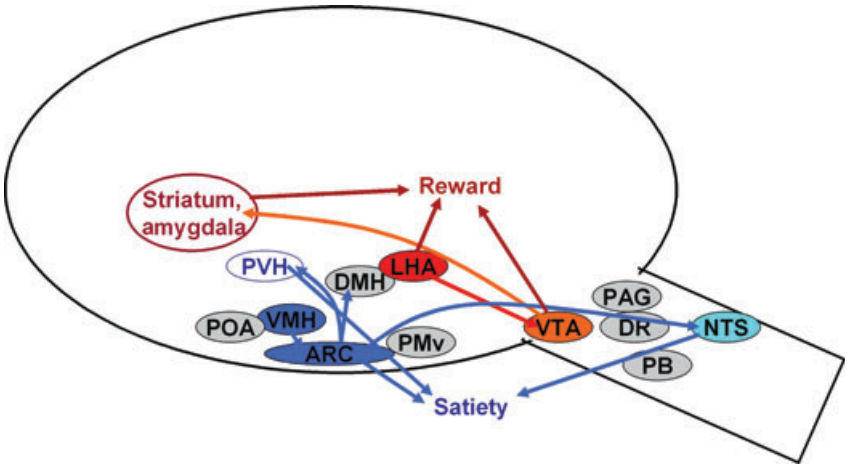

Figure 2 A distributed network of LRb-expressing leptin responsive neurones. In addition to the well-known arcuate nucleus of the hypothalamus (ARC), large populations of LRb-expressing neurones are found in a variety of other areas in the hypothalamus and brainstem (filled ovals). Regions that receive important projections from LRb neurones but that do not themselves contain large numbers of LRb neurones have white fills. Leptin action on LRb-expressing neurones in the ARC regulates neurones in the paraventricular hypothalamic nucleus (PVH), the dorsomedial hypothalamic nucleus (DMH) and the brainstem, including the nucleus of the solitary tract (NTS). These act, presumably along with LRb neurones within the DMH and NTS, to modulate satiety (blue circuits). The mesolimbic dopamine system (orange/red circuits) encodes incentive salience and reward; at the core of this system lie dopaminergic neurones in the ventral tegmental area (VTA) that project to the striatum and amygdala. Leptin regulates this system directly via LRb-expressing dopaminergic and non-dopaminergic neurones in the VTA, as well as via lateral hypothalamic (LHA) LRb neurones that project to the VTA to regulate dopamine production. Other areas containing less well understood LRb neurones (grey ovals) include the pre-optic area (POA), ventromedial hypothalamic nucleus (VMH), ventral premammillary nucleus (PMv), periaqeductal grey (PAG), dorsal raphe (DR) and parabrachial nucleus (PB).

receptor (MC4R) and the melanocortin-3 receptor (MC3R) (Huszar *et al.* 1997, Marsh *et al.* 1999, Butler *et al.* 2000, Chen *et al.* 2000, Ste *et al.* 2000, Butler & Cone 2002). LRb stimulates the synthesis of POMC and activates/depolarizes LRb/POMC neurones (Cowley *et al.* 2001, Elmquist *et al.* 2005, Morton *et al.* 2006). NPY is an orexigenic (appetite-stimulating) hormone that also suppresses the central LRb-mediated growth and reproductive axes (Clark *et al.* 1985, Erickson *et al.* 1996, Elmquist *et al.* 2005, Morton *et al.* 2006). AgRP is an antagonist of α-MSH/MC4R signalling as well as an inhibitor (inverse agonist) of endogenous MC4R activity (Ollmann *et al.* 1997, Elmquist *et al.* 2005, Morton *et al.* 2006). Leptin acts via LRb to inhibit NPY/AgRP neurones and suppress expression of these neuropeptides (Cowley *et al.* 2001, Elmquist *et al.* 2005, Morton *et al.* 2006). Thus, LRb signalling stimulates the production of anorectic neuropeptides and suppresses levels of orexigenic peptides. Conversely, a decrease or deficiency in leptin activity (e.g. during starvation and in *ob/ob* and *db/db* mice) stimulates appetite by suppressing synthesis of anorectic neuropeptides (e.g. POMC) and increasing expression of orexigenic peptides (e.g. NPY and AgRP).

We have previously examined the regulation of ARC neuropeptide expression and neuronal activity in *s/s* and *l/l* mice. Similar to *db/db* mice, *s/s* mice have decreased POMC mRNA levels in the hypothalamus (Bates *et al.* 2003). 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 neurones is appropriately suppressed in *s/s*, but not in *db/db* animals (Munzberg *et al.* 2007). These data suggest that LRb-STAT3 signalling is a crucial regulator of hypothalamic melanocortin action, and that dysregulated melanocortin signalling (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 neurones. In *l/l* mice, levels of POMC mRNA are similar to those found in wild-type mice, although levels of NPY and AgRP are decreased in *l/l* animals, suggesting increased LRb signalling in these animals and demonstrating that the regulation of these neuropeptides is independent of Tyr_{985}. Hence, non-STAT3, non-Tyr_{985} LRb signals are critical regulators of the LRb/NPY neurone.

LRb action beyond established ARC circuits

Although we now know a great deal about the mechanisms by which the ARC NPY/AgRP and POMC neurone function, numerous questions remain regarding the magnitude of their contributions to the regulation of

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feeding in response to leptin under physiological conditions (Boston *et al.* 1997, Ellacott & Cone 2006). Indeed, only 50% of ARC POMC neurones express LRb (Munzberg *et al.* 2003). Also, although ablation of AgRP neurones results in hypophagia and ablation of POMC or central melanocortin receptors results in severe obesity (Butler & Cone 2002, MacNeil *et al.* 2002), deletion of LRb from POMC neurones or the restoration of LRb in the ARC of *db/db* animals results in only modest alteration in body weight (Morton *et al.* 2003, Balthasar *et al.* 2004). Furthermore, although interference with LRb → STAT3 signalling in *s/s* mice results in dramatic hyperphagia and obesity, deletion of STAT3 in POMC and NPY/AgRP neurones only modestly impacts body energy homeostasis (Bates *et al.* 2003, Kaelin *et al.* 2006, Xu *et al.* 2006). Thus, while melanocortins and ARC POMC and NPY/AgRP neurones generally effect powerful appetitive signals, leptin is only one factor that regulates melanocortin action, and thus these neurones may not mediate the majority of the leptin-promoted anorectic signal. The aggregate leptin signal is probably mediated in concert with many other, currently uncharacterized, populations of LRb-expressing neurones. Indeed, some data suggest the existence of additional populations of ARC LRb neurones, including those that are modified by cre recombinase activity in rat insulin II gene prometer-Cre (RIP-Cre) mice (Cui *et al.* 2004, Smith *et al.* 2007).

Arcuate LRb neurones comprise only 15–20% of the total number of LRb-expressing neurones within the CNS (Leshan *et al.* 2006), and other populations of LRb neurones, including those in the VMH, and VTA clearly mediate an important component of leptin action (Dhillon *et al.* 2006, Fulton *et al.* 2006, Hommel *et al.* 2006). Thus, unlike insulin, which acts primarily on relatively homogeneous organs such as the liver and muscle, leptin regulates a broadly distributed network of LRb-expressing neurones in the brain in order to orchestrate an array of neural processes that range from neuroendocrine and sympathetic nervous system function, to satiety, and to the perception of food reward. It is becoming increasingly clear that various facets of leptin action are contributed to by discrete populations of LRb neurones, and that the distributed network of LRb neurones collectively mediates the totality of leptin action.

Leptin regulation of satiety

Satiety is the perception of fullness that indicates that no further food ingestion is needed. The ARC and VMH are defined as 'satiety centres' because lesions of either promote hyperphagia and obesity (Elmquist *et al.* 2005, Morton *et al.* 2006). The contributions of the ARC to leptin-mediated satiety (e.g. via LRb/POMC and LRb/NPY) is well characterized, as discussed above. VMH LRb neurones also contribute to satiety via excitatory projections onto ARC POMC neurones (Pinto *et al.* 2004, Sternson *et al.* 2005). The density of these projections is dynamically regulated by leptin availability (i.e. fasting or fed states) demonstrating the exquisite sensitivity of the VMH to physiological changes. At least one subpopulation of VMH LRb neurones, those co-expressing the transcription factor SF-1, is activated by leptin and contribute to leptin-mediated satiety (Dhillon *et al.* 2006).

Several areas of the brainstem, including the NTS and the area postrema, are also important for satiety (Elmquist *et al.* 2005, Grill 2006, Morton *et al.* 2006). The brainstem integrates numerous inputs from the gut [including those from vagal afferents and the anorexigenic gut peptides glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK)], as well as the hypothalamus, to modulate satiety (Grill 2006). Leptin acts synergistically with signals of gastric distension, GLP-1 and CCK to regulate the neurones of the NTS, and thus contribute to satiety (Morton *et al.* 2005, Williams *et al.* 2006, Huo *et al.* 2007). The NTS and other brainstem regions associated with satiety contain LRb neurones, and direct leptin action on brainstem LRb-expressing neurones as well as indirect leptin action via ARC LRb neurones that project to the brainstem may contribute to the satiety effects of leptin (Elmquist *et al.* 1998a, Grill *et al.* 2002, Ellacott *et al.* 2006a,b, Grill 2006, Leshan *et al.* 2006, Huo *et al.* 2007).

Leptin regulation of the mesolimbic dopamine system

In addition to satiety, the rewarding properties of food contribute to feeding (Kelley & Berridge 2002, Figlewicz *et al.* 2006). For example, after ingestion of a meal you may feel satiated (full), but may go on to eat dessert because it seems appealing and tasty. Thus, the reward value of food induces ingestion beyond what is needed for satiety. Leptin regulates the reward value placed on food (as well as of other addictive substances, such as drugs of abuse) (Fulton *et al.* 2004, Carr 2006, Figlewicz *et al.* 2006). 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 (Figlewicz 2003, Figlewicz *et al.* 2003, Fulton *et al.* 2006, Hommel *et al.* 2006). The core of the mesolimbic DA system lies in a set of DA neurones in the VTA that project forward to innervate the striatum (nucleus accumbens, caudate, putamen), amygdala and prefrontal cortex. 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

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the incentive salience of food and other natural rewards (Kelley & Berridge 2002, Nestler 2005). The relationship between the DA system and energy homeostasis has been often described but is poorly understood. For example, side effects of antipsychotic drugs (that act in part as mixed DA antagonists) include weight gain and metabolic disease (Bergman & Ader 2005).

While ARC LRb neurones do not project to the VTA, systemic leptin administration modulates food reward, suggesting a different locus for the regulation of DA signalling (Figlewicz *et al.* 2006). A number of groups have reported the presence of LRb-expressing VTA DA neurones and demonstrated the ability of leptin to alter the physiology of the DA system (Figlewicz 2003, Figlewicz *et al.* 2003, Fulton *et al.* 2006, Hommel *et al.* 2006). Thus, leptin may act directly upon VTA LRb neurones to regulate DA synthesis and release in the striatum and amygdala.

The lateral hypothalamic 'feeding' centre interacts extensively with the mesolimbic DA system to regulate motivation and reward, including food reward (Kelley & Berridge 2002, DiLeone *et al.* 2003, Fulton *et al.* 2004). Furthermore, states of nutritional deficiency modulate rewarding intrahypothalamic self-stimulation (IHSS) in the perifornical area of the LHA (Fulton *et al.* 2004). LHA orexin neurones project to the VTA and regulate drug and food-associated reward signalling, and LHA melanin-concentrating hormone (MCH) neurones interact with the striatum (DiLeone *et al.* 2003, Harris *et al.* 2005).

The LHA is another important locus of leptin action on the DA system. Leptin inhibits the activity of orexin neurones and reduces the expression of the LHA neuropeptide MCH, as well as attenuating IHSS (Qu *et al.* 1996, Fulton *et al.* 2000, 2004, Yamanaka *et al.* 2003). Inhibition of OX and MCH signalling via their respective receptor antagonists inhibit food intake and modulate DA signalling in rodent models similar to leptin, and these pathways are thus potential drug targets for anxiety and weight loss. We have also identified a novel population of LRb-expressing neurones in the LHA that project to the VTA to regulate the mesolimbic DA system (our unpublished data). As in the ARC, these LHA LRb neurones are composed of multiple different subpopulations of neurones that are divergently regulated by leptin and other nutritional factors. 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 probably regulate the incentive salience of food.

Summary

The totality of leptin action neither reflects the output of a single intracellular signal nor that of a single (e.g. ARC) neural circuit, but rather integrates multiple LRb-stimulated signals acting in concert upon a widely distributed and heterogeneous array of LRb-expressing neurones. The spatial diversity along with the distinct complements of neurotransmitters employed by these myriad LRb-expressing neural populations suggests the divergent function of many subpopulations of LRb neurones even within the discrete LRb-expressing brain regions. It will be important to understanding the phenotype (neural inputs and outputs; neurotransmitters) of each distinct population of LRb-expressing neurones and the roles for the various LRb-mediated signals in each set of neurones in order to truly understand the mechanisms of leptin action. This information is likely to reveal not only novel information about leptin action, but also potential novel targets for the regulation of feeding and the treatment of obesity.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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