

Leptin Receptor Signaling and Action in the Central Nervous System

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Abstract

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The increasing incidence of obesity in developed nations represents an ever-growing challenge to health care by promoting diabetes and other diseases. The discovery of the hormone, leptin, a decade ago has facilitated the acquisition of new knowledge regarding the regulation of energy balance. A great deal remains to be discovered regarding the molecular and anatomic actions of leptin, however. Here, we discuss the mechanisms by which leptin activates intracellular signals, the roles that these signals play in leptin action in vivo, and sites of leptin action in vivo. Using “reporter” mice, in which LRB-expressing (long form of the leptin receptor) neurons express the histological marker, β -galactosidase, coupled with the detection of LRB-mediated signal transducer and activator of transcription 3 signaling events, we identified LRB expression in neuronal populations both within and outside the hypothalamus. Understanding the regulation and physiological function of these myriad sites of central leptin action will be a crucial next step in the quest to understand mechanisms of leptin action and energy balance.

Key words: hypothalamus, transgenic animals, leptin action

Introduction

Leptin is produced in proportion to fat stores; adequate leptin levels serve to communicate the repletion of body

energy stores to the central nervous system (CNS)¹ to suppress food intake and permit energy expenditure (1–3). Conversely, lack of leptin signaling caused by 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 a phenotype of reduced energy expenditure reminiscent of the neuroendocrine starvation response (1,2,4). Leptin also regulates insulin sensitivity and glucose homeostasis by two mechanisms: one by controlling energy balance and body fat (increased body adiposity leads to insulin resistance) and the other through an adiposity-independent pathway mediated by the CNS (5,6).

LR Signaling

The long form of the LR, LRB, mediates intracellular signaling and is, thus, crucial for leptin action (3,7,8). LRB initiates signals by activating a non-covalently associated Jak family tyrosine kinase (JAK2) that autophosphorylates on numerous tyrosine residues as well as phosphorylating tyrosine residues on LRB during leptin stimulation (8–10). The phosphorylation of tyrosine at position 985 (Tyr₉₈₅) on LRB recruits the tyrosine phosphatase, tyrosine phosphatase 2, which thereby mediates a portion of the activation of the canonical p21ras → extracellular signal-regulated kinase (ERK) signaling pathway in cultured LRB-expressing cells. Tyr₉₈₅ also plays an important role in LRB signal attenuation by binding the inhibitory suppressor of cytokine signaling-3 (SOCS3) molecule (11).

Phosphorylation of Tyr₁₁₃₈ recruits the latent transcription factor, signal transducer and activator of transcription 3 (STAT3), to the LRB/JAK2 complex, resulting in the tyrosine phosphorylation and nuclear translocation of STAT3 to effect its regulation of transcription (9,10). Among other genes, STAT3 activates the transcription of the neuropep-

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¹ Nonstandard abbreviations: CNS, central nervous system; LR, leptin receptor; JAK2, Jak family tyrosine kinase; ERK, extracellular signal-regulated kinase; SOCS3, suppressor of cytokine signaling-3; STAT3, signal transducer and activator of transcription 3; POMC, proopiomelanocortin; PI3K, phosphatidylinositol 3-kinase; ARH, arcuate nucleus; ISH, in situ hybridization; IHC, immunohistochemistry; IF, immunofluorescence; β -gal, inducible β -galactosidase; VMH, ventromedial hypothalamus.

ptide, proopiomelanocortin (POMC), and the signaling inhibitor, SOCS3 (10,12,13). Tyr₁₀₇₇ and Tyr₁₁₃₈ on LRb cooperate to mediate activation of STAT5. JAK2 tyrosine phosphorylation during LRb stimulation mediates some signals independently of tyrosine phosphorylation sites on LRb (e.g., a portion of ERK activation), but the exact nature and function of these signals is not yet fully understood (10).

LRb Signaling in the Regulation of Physiology

Roles for a number of intracellular signaling pathways have been examined in the regulation of energy balance. The pharmacological inhibition of phosphatidylinositol 3-kinase (PI3K) blocks the suppression of feeding by leptin, suggesting that PI3K signaling contributes to the regulation of energy balance by leptin (14). Indeed, activation of the PI3K-regulated Akt/protein kinase B pathway in the hypothalamus mediates anorexia (15). Although the deletion of SHP-2 or STAT3 from forebrain neurons results in over-feeding and obesity (16,17), it is not clear whether this obesity stems from perturbations in leptin signaling, per se, or is the less-specific consequence of the widespread deletion of these promiscuous signaling molecules. The role of LRb→STAT3 signaling in energy balance has been clearly established by the study of rodents containing a homologous replacement of LRb by a receptor mutant for Tyr₁₁₃₈, the STAT3 binding site (13,18,19), however. The LRb Tyr₁₁₃₈→STAT3 signal is required for the control of feeding and energy expenditure by leptin, although this signal is not absolutely required for the regulation of growth, reproduction, immune function, and glycemic control. Thus, another signaling pathway must mediate these STAT3-independent functions of LRb.

Leptin Action in the CNS

Many of the actions of leptin are attributable to effects in the CNS, particularly in the basomedial hypothalamus, including the arcuate nucleus (ARH) (2,20). Thus, LRb signaling stimulates the elaboration of anorectic neuropeptides and suppresses the action of orexigenic peptides in the ARH. The finding that the ARH-specific expression of LRb action ameliorates appetite and decreases adiposity in rodents lacking LRb suggests the importance of ARH leptin action for energy balance (21,22). An important component of the adiposity-independent regulation of glucose metabolism by leptin is also mediated through the ARH (22). It is clear, however, that ARH leptin action represents only one component of leptin action on energy balance, because the deletion of LRb from POMC neurons results in a relatively modest overweight phenotype, and the restoration of LRb expression specifically in the arcuate nucleus of *db/db* mice reverses only a portion of the hyperphagia and obesity of these animals (22,23). Other populations of leptin-respon-

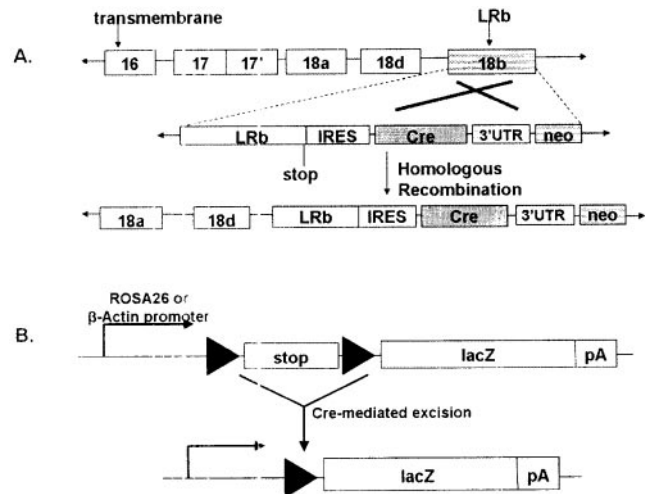


Figure 1: *Lepr*^{Cre}; *iβ-gal* reporter mice. (A) We used genetic targeting in mouse embryonic stem cells (ES cells) to insert an IRES element plus the coding sequences for cre recombinase into the 3'-untranslated region of exon 18b, the LRb exon, placing cre expression under the control of the mRNA encoding LRb. Correctly targeted ES cells were used to generate mice expressing cre in LRb-expressing cells (*lepr*^{Cre} mice). To produce experimental animals, we mated LRb-cre mice with ROSA26-*iβ-gal* or actin-*iβ-gal* (B) mice, in which a floxed transcription blocker precedes *lacZ*, such that *β-galactosidase* expression is only permitted after Cre-mediated recombination/removal of the transcription blocker. In the ROSA26-*iβ-gal* strain, expression is driven by the ROSA26 promoter. In actin-*iβ-gal* mice, expression is driven by the chicken *β-actin* promoter. In *Lepr*^{Cre}; *iβ-gal* mice, cre-mediated excision of the transcription blocker results in *β-gal* expression in cells expressing LRb and in which the transgene promoter is active.

sive (i.e., LRb-expressing) neurons within the hypothalamus and elsewhere in the CNS remain uncharacterized.

Genetic Reporter System for LRb Expression and Action in the CNS

While several assays detect LRb expression within the CNS, each of these has limitations that prompted us to seek a more facile system for identifying LRb-expressing neurons. In situ hybridization histochemistry (ISH) for LRb is difficult, more so if done in combination with immunohistochemistry (IHC) or immunofluorescence (IF) for other proteins. Furthermore, the detection of leptin-induced STAT3 phosphorylation by IHC/IF or of leptin-induced SOCS3 expression by ISH, while robust, requires prior treatment with leptin, limiting the use of this method for functional assays in the absence of leptin or LRb. We generated mice in which an internal ribosome entry site plus the coding sequence for the cre recombinase were genetically targeted into the 3'-non-coding region of the LRb-specific exon within the leptin receptor gene (*lepr*^{Cre}

Table 1. Sites of observed β -gal immunoreactivity in reporter mouse brains. Relative densities of β -gal immunoreactive cells in *lepr^{Cre}*; ROSA26-*i* β -gal and *lepr^{Cre}*; and Actin-*i* β -gal

	ROSA26	Actin	Functions of the Region
FOREBRAIN			
Hippocampus			Learning; memory
dentate gyrus	–	+++	
CA1	–	+++	
CA2	–	+++	
CA3	–	+++	
Hypothalamus			
arcuate nucleus	+++		Feeding; Energy homeostasis
ventromedial hypothalamus	+++	+	Satiety
dorsomedial hypothalamus	+++	–	Feeding; Energy homeostasis; Reproduction
lateral hypothalamus	+++	–	Hunger, reward, attention
pre-mammillary nucleus, ventral part	+++	–	Reproduction; Anxiety; Fear conditioning
MIDBRAIN			
dorsal raphe	+++	–	Cognition; Arousal; Stress response; Feeding
HINDBRAIN			
Pons			
reticulotegmental nucleus of the pons	–	+	Oculomotor circuit component; Arousal
rostral periolivary region	–	+	Auditory functions?
Medulla			
nucleus of the solitary tract	++	–	Energy homeostasis; Cardiorespiratory responses

+++ , high; ++ , medium; + , low; – , none above background.

mice; Figure 1A). The LRb coding region of these mice is fully intact, suggesting that energy homeostasis in these animals should be normal; indeed, we have not observed any body weight phenotype in animals homozygous for *lepr^{Cre}* compared with control animals. We crossed these *lepr^{Cre}* animals with mice containing transgenes encoding cre-inducible β -galactosidase expression (*i* β -gal), wherein cre recombinase expression removes a transcription-blocking cassette that is flanked by LoxP sites, enabling the expression of downstream β -gal coding sequences (Figure 1B). We used two strains of *i* β -gal mice, ROSA26-*i* β -gal (24) and actin-*i* β -gal (25), in which the promoters for ROSA26 and chicken β -actin drive transgene expression, respectively. Thus, in the *lepr^{Cre}*; *i* β -gal mice, we expect excision of the transcription blocker and subsequent β -gal expression in neurons that express high levels of LRb mRNA and in which the promoter for the *i* β -gal transgene operates strongly (Figure 1B).

The immunofluorescent detection of β -gal in the brains of the *lepr^{Cre}*; *i* β -gal mice revealed positive cells in the ARH and ventromedial hypothalamus (VMH) in both reporter strains. Specific to the actin-*i* β -gal mice, we observed abundant β -gal-expressing cells in the hippocampus (data

not shown), as well as smaller numbers of cells in the pons and VMH. In ROSA26-*i* β -gal reporter animals, positive cells were abundant in the ARH, pre-mammillary nucleus, dorsomedial hypothalamus, lateral hypothalamus, VMH, and dorsal raphe. No β -gal expression was detected in either *i* β -gal strain in the absence of the *lepr^{Cre}* allele. The distribution of positive cells in both reporter strains is summarized in Table 1. The distribution of β -gal in these reporter animals is consistent with the previous detection of LRb mRNA and with a potential functional role for leptin action in these regions (26).

To validate the expression of functional LRb in these β -gal-positive neurons, we performed double-label IF to detect both β -gal and pSTAT3 in leptin- or phosphate-buffered saline-treated *lepr^{Cre}*; ROSA26-*i* β -gal reporter mice (Figure 2). We observed cells positive for both pSTAT3 and β -gal in the ARH, pre-mammillary nucleus, dorsomedial hypothalamus, VMH, lateral hypothalamus, and dorsal raphe. While not all neurons with leptin-induced pSTAT3 were labeled by β -gal expression, almost all cells expressing β -gal were also positive for pSTAT3. Thus, whereas populations of LRb neurons exist that are not labeled by this genetic method, the β -gal-positive neurons

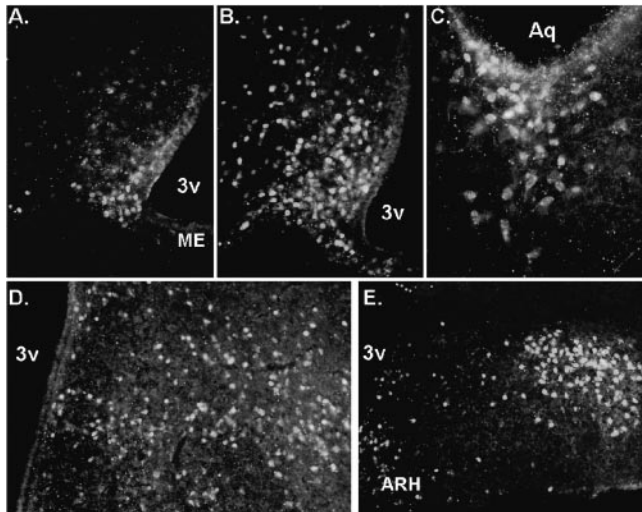


Figure 2: Immunofluorescence for β -gal and pSTAT3 in leptin-treated *Lepr^{Cre}*; ROSA26- $i\beta$ -gal mice. Adult (8 to 10 weeks) mice were injected intraperitoneally with vehicle (A) or leptin (5 mg/kg; B–E). One hour after injection, animals were anesthetized, and brains were fixed by intracardiac perfusion with ice-cold 4% paraformaldehyde in 0.9% sodium chloride and processed for pSTAT3 IF as described previously (28) plus β -gal IF. Sections were mounted on gelatin-coated slides using ProLong Gold antifade medium (Molecular Probes) and visualized using a fluorescence microscope. Representative images from *lepr^{Cre}*; ROSA26- $i\beta$ -gal mice show leptin-induced pSTAT3 immunoreactivity (nuclear) colocalized with β -gal- (cytoplasmic) in cells of the ARH (A and B), DR (C), DMH (D), and PMV (E). 3v, third cerebral ventricle; ME, median eminence.

in these reporter mice express functional LRb and respond to leptin.

This method for identifying LRb-expressing neurons should facilitate the dissection of the regulation and function of LRb-expressing populations of neurons throughout the brain. Understanding the biology of these novel populations of leptin-responsive neurons will be crucial for our understanding of the mechanisms regulating energy balance and in designing future therapies for obesity.

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