Published in final edited form as:

Brain Res. 2010 July 23; 1345: 146–155. doi:10.1016/j.brainres.2010.05.042.

Innervation of Skeletal Muscle by Leptin Receptor-Containing Neurons

Tanja Babic², Megan N. Purpera¹, Bruce W. Banfield³, Hans-Rudolf Berthoud¹, and Christopher D. Morrison¹

¹Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA

²Department of Neural and Behavioral Sciences, Penn State College of Medicine, Hershey, PA 17033

³Department of Microbiology & Immunology, Queen's University, Kingston, Ontario K7L 3N6, Canada

Abstract

In addition to suppressing food intake, leptin reduces body adiposity by altering metabolism within peripheral tissues such as adipose tissue and muscle. Recent work indicates that leptin action within the brain is sufficient to promote glucose uptake and increase fat oxidation within skeletal muscle, and that these effects are dependent on the sympathetic nervous system. To identify neuronal circuits through which leptin impacts skeletal muscle metabolism, we used LepRb-GFP reporter mice in combination with muscle specific injection of a mRFP-expressing pseudorabies virus (PRV), which acts as a transynaptic retrograde tracer. Consistent with previous observations in the rat, muscle-specific PRV injection lead to labeling within multiple areas of the hypothalamus and brainstem. However, the only areas in which PRV and LepRb colocalization were detected was within the brainstem nucleus of the solitary tract (NTS) and the hypothalamic retrochiasmatic area. Within the NTS $28.5 \pm 9.4\%$ of PRV-positive neurons contained LepRb-GFP, while in the RCH $37 \pm 1.7\%$ of PRV neurons also contained LepRb. In summary, these data clearly implicate the NTS and RCH as key sites through which brain leptin impacts skeletal muscle, and as such provide an anatomical framework within which to interpret physiological data indicating that leptin acts in the brain to influence metabolism within skeletal muscle.

Keywords

Leptin receptor; muscle; sympathetic; pseudorabies virus

INTRODUCTION

Leptin acts in the brain to regulate body weight and adiposity, most notably by acutely inhibiting food intake (Berthoud and Morrison, 2008). However, leptin also exerts clear effects on energy expenditure, thermogenesis, and substrate oxidation, and these latter

© 2010 Elsevier B.V. All rights reserved.

Corresponding Author: Christopher D. Morrison, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808, Phone: (225) 763-3145, Fax: (225) 763-0260F, morriscd@pbrc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

effects are mediated at least in part by a leptin-dependent regulation of autonomic outflow (Collins et al., 1996; Haque et al., 1999; Minokoshi et al., 2002; Montanaro et al., 2005; Morgan et al., 2008; Rahmouni et al., 2002). For instance, leptin increases sympathetic nervous system (SNS) outflow to brown adipose tissue, increasing norepinephrine turnover and UCP1 expression (Collins et al., 1996; Commins et al., 2000; Haynes et al., 1997; Morrison, 2004; Scarpace and Matheny, 1998). Similarly, leptin activates SNS outflow to white adipose tissue, promoting lipolysis and inhibiting its own expression (Commins et al., 2000). Lastly, leptin injection into the lateral ventricles or into the arcuate nucleus increases lumbar and renal sympathetic nerve activity, arterial pressure and heart rate (Dunbar et al., 1997; Haynes et al., 1997; Rahmouni and Morgan, 2007). These data collectively indicate that a key feature of leptin action is a coordinated regulation of sympathetic outflow to peripheral tissues.

The sympathetic nervous system also innervates skeletal muscle, altering vascular tone and blood flow (Kerman et al., 2000; McAllen and Dampney, 1990) as well as changes in thermogenesis and metabolism (Minokoshi and Kahn, 2003; Nogueiras et al., 2007; Nonogaki, 2000; Wijers et al., 2008). Interestingly, leptin appears to regulate muscle metabolism via a central circuit, as injections of leptin into the brain, and particularly the hypothalamus, increase SNS outflow to muscle (lumbar) (Dunbar et al., 1997), stimulate glucose uptake (Minokoshi et al., 1999), and promote fatty acid oxidation (Minokoshi et al., 2002), and these metabolic effects of leptin are dependent on the SNS (Haque et al., 1999; Minokoshi et al., 2002). Taken together, these observations are consistent with leptin acting within the brain to orchestrate metabolic changes within peripheral tissues which serve to both increase insulin sensitivity and glucose metabolism (German et al., 2009; Kamohara et al., 1997; Morton et al., 2005) and reduce lipid accumulation and steatosis (Lee et al., 2001).

While these effects of leptin are relatively well established, the mechanism by which leptin regulates sympathetic outflow to muscle is less clear. Some components of the central circuitry that controls sympathetic outflow to the muscle have been identified (Kerman et al., 2003; Kerman et al., 2006; Lee et al., 2007), and injections of pseudorabies virus (PRV), a transsynaptic retrograde tracer, into the hindlimb muscles resulted in infection of neurons in the regions of the brainstem and the hypothalamus that are known to affect sympathetic outflow (Kerman et al., 2003; Kerman et al., 2006; Lee et al., 2007). However, the specific sites in which leptin acts to regulate the SNS remain unclear. To address this question, the current work targets a PRV-based transsynaptic retrograde tracer to the muscle of mice bearing green fluorescent protein expression within long-form leptin receptor (LepRb-GFP) containing neurons. The result is the identification of two unique populations of neurons that bear leptin receptors and project transsynaptically to skeletal muscle.

RESULTS

Distribution of PRV-labeled neurons in the brainstem

4.5 days after PRV injections into the gastrocnemius muscle, PRV-containing neurons were observed in the rostral and caudal ventrolateral medulla (RVLM and CVLM, respectively), ventromedial medulla, including the Raphe magnus (RMg) and gigantocellular reticular formation pars alpha (GiA) and in the raphe pallidus (RPa). After a survival period of 5.5 days, a greater number of PRV-containing neurons were observed in the ventrolateral and ventromedial medulla, and PRV-positive neurons were observed in the raphe obscurus, locus coeruleus (LC), reticular formation, superior olivary nucleus (SPO) and the nucleus of the solitary tract (NTS) (Fig. 1). In the NTS, PRV-positive neurons were observed primarily in the ventrolateral and intermediate subnuclei (Fig. 2). The number and the distribution of PRV-containing neurons in the brainstem after a 6.5 day survival period was not different from that after a 5.5 day survival period.

Colocalization of PRV with leptin receptor in the NTS

Within the brainstem, leptin receptors are expressed principally within a relatively small population of neurons within the NTS, and PRV infection was not detected within this brain area until 5.5 days post-infection. Based on quantitative analysis of every third section throughout the NTS (5.5 days postinfection), 19.7 ± 5.1 neurons were positive for PRV, 20.0 ± 3.1 neurons contained LepRb-GFP and 4.7 ± 0.9 neurons were double labeled. These numbers translate into $28.5 \pm 9.4\%$ of PRV-positive neurons containing LepRb-GFP, whereas $23.2 \pm 2.6\%$ of LepRb-GFP-containing neurons also contained PRV (Fig. 3). Double labeled neurons were localized in the caudal NTS, primarily in the ventrolateral and intermediate subnuclei (Fig. 2 and 3). No double-labeling was detected within any other brainstem structure.

Distribution of PRV-labeled neurons in the hypothalamus

4.5 days after PRV injections into the gastrocnemius muscle, PRV-labeled neurons were observed in the paraventricular nucleus of the hypothalamus (PVH), with only a small number of neurons in the lateral hypothalamus (LHA). 5.5 days after PRV injections, a greater number of PRV-containing neurons were observed in the PVH and the LHA, and labeled neurons were also detected within the retrochiasmatic nucleus (RCH), ventromedial nucleus of the hypothalamus (VMH), arcuate nucleus (Arc) and the dorsomedial hypothalamus (DMH; Fig. 4).

Colocalization of PRV with leptin receptor in the hypothalamus

A large number of GFP positive neurons were observed in the LHA, Arc, RCH, VMH and DMH, consistent with previous observations (Leshan et al., 2009). In addition, a small number of GFP-positive neurons were observed in the PVH (Figure 5). Yet despite the presence of significant populations of both PRV and LepRb neurons within these areas, double labeled neurons were only detected within the RCH, which were localized between 0.94 and 1.22 mm caudal to bregma (Figure 6). In the RCH, 48.3 ± 15.3 neurons contained PRV, 108.3 ± 9.1 contained LepRb-GFP and 17.7 ± 5.2 neurons were double labeled. Thus $37.4 \pm 1.7\%$ of neurons that contained PRV also contained LepRb-GFP, whereas $17.0 \pm 5.5\%$ of GFP-positive neurons also contained PRV (Figure 7).

DISCUSSION

While a primary effect of brain leptin signaling is a reduction in food intake, available data increasingly indicate that leptin also induces coordinated changes within energy metabolism and substrate oxidation within peripheral tissues. For instance, leptin-deficient ob/ob mice remain obese even when food intake is reduced to normal levels. Leptin treatment in these pair-fed ob/ob mice effectively reduces body adiposity (Coleman, 1985; Levin et al., 1996), indicating that leptin can effectively alter body composition in the absence of any change in food intake. These observations suggest that leptin has additional impacts on energy metabolism and substrate oxidation, and much work has focused on leptin's ability to increase sympathetic outflow to brown adipose tissue (Collins et al., 1996; Commins et al., 2000; Haynes et al., 1997; Morrison, 2004; Scarpace and Matheny, 1998). However, leptin's ability to reduce body adiposity and promote fat oxidation occurs even in the absence of UCP1-dependent BAT thermogenesis (Ukropec et al., 2006). Thus while the thermogenic regulation of body temperature by leptin appears to involve BAT, leptin alters metabolism and decreases body adiposity via mechanisms that are in addition to the suppression of food intake and the activation of BAT (Ukropec et al., 2006).

Based on these and other observations, we focused on the neuronal circuits that likely contribute to leptin-dependent regulation of muscle metabolism, particularly via activation

of the sympathetic nervous system. Several recent studies indicate that central injection of leptin is sufficient to alter glucose and lipid metabolism within muscle, and that these effects are dependent on sympathetic innervation (Dunbar et al., 1997; Haque et al., 1999; Minokoshi et al., 1999; Minokoshi et al., 2002). Currently the neuroanatomical pathways that mediate these leptin-dependent effects are unclear. To address this question, the current study combines two well-established reporter and tracer techniques. The first is a genetic approach in which green-fluorescent protein (GFP) is expressed locally within neurons that contain the long (signaling) form of the leptin receptor (LepRb(Leininger et al., 2009; Leshan et al., 2006). The second is the use of a red-fluorescent protein bearing pseudorabies virus (PRV) as a multisynaptic retrograde tracer (Banfield et al., 2003). PRV based tracing has been used extensively to delineate autonomic innervation of brown and white adipose tissue (Bamshad et al., 1998; Bamshad et al., 1999; Oldfield et al., 2002), and recent work in the rat has described labeling in the hindbrain and forebrain following PRV injections into skeletal muscle (Kerman et al., 2003; Kerman et al., 2006). Therefore, the primary goal of the current work was to determine the degree of anatomical similarity between mice and rats following muscle specific PRV injection, and to determine whether specific populations of leptin receptor expressing neurons were labeled by muscle-derived PRV.

Our observations confirmed previous work demonstrating the existence of a small, discrete population of leptin receptor expressing neurons within the nucleus of the solitary tract (Leshan et al., 2006; Myers et al., 2009). Despite this rather small population, previous experiments demonstrate that local injection of leptin into this brain area is sufficient to decrease food intake (Grill et al., 2002; Grill and Hayes, 2009). A small percentage of these NTS leptin receptor neurons were also labeled by muscle-derived PRV, indicating that some of these neurons project transynaptically to muscle and may contribute to leptin-dependent changes in muscle metabolism. Similar to prior studies in the rat, muscle-derived PRV labeling was detected in many additional areas of the brainstem, including early detection in the rostral ventrolateral medulla (RVLM), A5 noradrenergic cell group, rostral ventromedial medulla (RVMM), raphe pallidus (Rpa), locus coeruleus (LC) and locus subcorelueus (SC) (Kerman et al., 2003; Kerman et al., 2006; Lee et al., 2007). It should be noted that PRV labeling within the NTS appears relatively later than other areas of the brainstem. As such, it is possible that these leptin-receptor bearing PRV neurons within the NTS do not project directly to the IML, but instead transmit information their information indirectly via other sites within the brainstem, particularly the medullary reticular formation.

Within the hypothalamus, leptin receptor containing neurons were detected across a much wider area as compared to the brainstem, with significant populations detected within the ARC, LHA, RCH, and DMH. PRV infected neurons were primarily detected within the paraventricular nucleus (PVH) and the lateral hypothalamus (LHA), with a smaller number of infected neurons in the dorsomedial (DMH), posterior hypothalamus (PH), and retrochiasmatic area (RCH), again consistent with prior experiments of muscle PRV injection in rats (Kerman et al., 2006). Surprisingly, the only area of substantial colocalization between leptin receptor and muscle-derived PRV was the retrochiasmatic area. The RCH is well known to contain neurons that project directly to the IML of the spinal cord, and at least a subpopulation of these neurons have been shown to be leptin sensitive (Elias et al., 1998). Thus the presence of colocalization within the RCH was not surprising. What was surprising was the lack of colocalization within other hypothalamic areas, particularly the DMH and LHA, considering that relatively large populations of both PRV and leptin receptor containing neurons were detected at each of these sites. However, prior experiments within the LHA at least indicate that PRV injections into muscle or BAT leads to the labeling of a large percentage of MCH and orexin neurons (Kerman et al., 2007; Oldfield et al., 2002), neither of which appear to express leptin receptors (Leininger et al.,

2009). Thus leptin receptor expressing neurons in the LHA appear to represent a population that is distinct from currently described orexigenic/autonomic populations.

The current data provide the first anatomical description of the relationship between brain leptin receptor expressing neurons and skeletal muscle innervation. However, a few technical considerations should be recognized. First, while we hypothesize that the described PRV labeling reflects sympathetic innervation, motor innervation to skeletal muscle was left intact in our studies, and it is therefore possible that some of the PRV labeling detected in these studies reflects motor and not sympathetic pathways. However, the areas of focus in the current work, particularly the NTS and RCH, are well-described locuses for sympathetic outflow while not being associated with motor outflow, and previous work utilizing PRV injection into BAT or motor denervated skeletal muscle produces labeling that is highly consistent with the current observations. Second, it is also possible that the current experiments underestimated the number of neurons, particularly double-labeled neurons, due to the possibility that PRV infection produces alterations in cellular physiology that reduce neuronal peptide expression (Ray and Enquist, 2004; Song et al., 2005). While previous experiments have used other markers of leptin signaling (Stat3 phosphorylation) to corroborate experiments using the GFP reporter mouse, this approach was not feasible for the current experiments because PRV infection induced phospho-Stat3 even within non-leptin receptor containing neurons. Lastly, while our data definitively demonstrate that neurons within the RCH and NTS contain leptin receptors and project to skeletal muscle, it remains possible that populations may exist within other brain areas that were not detected here. Recent experiments indicate that a distinct population of leptin receptor neurons are also expressed within the preoptic area (Scott et al., 2009). The POA is also known to express PRV derived from white and brown adipose tissue injection (Bamshad et al., 1998; Bamshad et al., 1999), suggesting a high likelihood that this area also contains PRV following injection into skeletal muscle. Unfortunately, methodological issues with tissue handling precluded the assessment of the preoptic area in the current experiments, and therefore it remains possible that populations of PRV-labeled, leptin receptor-containing neurons may exist within the POA or alternative brain areas that we have not yet identified specifically.

In summary, the current work demonstrates the existence of distinct populations of leptin receptor containing neurons within the retrochiasmatic area and nucleus of the solitary tract which project transynaptically to skeletal muscle, likely via the sympathetic nervous system. As such, the current work provides an anatomical basis for prior physiological studies demonstrating that leptin action within the brain influences glucose and lipid metabolism in muscle via a mechanism dependent on the sympathetic nervous system.

Experimental Procedures

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee and conformed to the guidelines of the National Institutes of Health. Experiments were conducted in male and female LepRb-GFP transgenic mice (Leshan et al., 2009), which were group housed under standard laboratory conditions (12:12 hr light cycle). Food and water were available *ad libitum*. All experiments involving PRV virus were conducted under Biosafety Level 2 containment. Experiments were based on 3 animals per group for each infection duration.

Pseudorabies Virus Injection

Animals were anesthetized with ketamine/acepromazine/xylazine (80/1.6/4.0 mg/kg,SC), and the left gastrocnemius muscle was exposed and injected with pseudorabies virus expressing monomeric red fluorescent protein (PRV 614, (Banfield et al., 2003)). Animals were injected with 0.5 ul of 8.4×10^8 plaque forming units/ml, using a 5 ml-Hamilton syringe fitted with a 26-gauge needle (Berthoud et al., 2005; Zheng et al., 2005). PRV was injected into six sites per muscle, and the needle was held in place for 3 minutes after the injection before being removed.

Tissue Preparation

After 4.5, 5.5 or 6.5 day survival time, mice were deeply anesthetized and perfused with heparanized 0.9% saline, followed by 4% paraformaldehyde. The brains were extracted, blocked and post-fixed for a minimum of 2 hr. Tissue was then cryoprotected in 15% sucrose in 0.1M phosphate buffered saline (PBS) overnight at 4°C. Coronal sections of the brainstem and the hypothalamus were cut on a cryostat at 20 um for brainstem and 30 um for hypothalamus. Sections were separated into six series and stored in a cryoprotectant solution (50% PBS, 30% ethylene glycol, 20% glycerol) at -20°C.

Immunohistochemistry

One series of sections through the brainstem and the hypothalamus from each animal was processed for GFP immunohistochemistry. With appropriate washes between incubations, free-floating sections were rinsed with fresh 0.5% sodium borohydride in PBS followed by pretreatment in a blocking solution of 5% normal donkey serum in PBS with 0.5% Triton X-100. Sections were incubated in goat anti GFP antibody diluted 1:500 (AbCam, Cambridge, MA) overnight. Tissue was then incubated in biotinylated donkey anti-goat IgG (1:250; Jackson ImmunoResearch; West Grove, PA) for 60 min, followed by 1% streptavidin-conjugated AlexaFluor488 (Invitrogen; Carlsbad, CA). Sections were mounted out of glycerol onto glass microscope slides and covered with glycerol.

Data Analysis

Neurons positive for PRV and immunoreactive for GFP were counted visually using a fluorescent microscope and 20× objective. The number of single- or double-labeled neurons was counted bilaterally on every third section of the brainstem and the hypothalamus. The total number neurons was calculated for each structure of the brainstem and the hypothalamus that contained PRV and GFP. The location of labeled neurons was mapped onto a standard set of drawings of the brainstem and the hypothalamus. Nomenclature of Paxinos (Paxinos and Franklin, 2001) was used.

Acknowledgments

LepRb-GFP reporter mice were provided as a kind gift from Dr. Martin G. Myers, Jr. This work was supported in part by National Institutes of Health grant P20-RR021945 to CDM, and by the Metabolic and Genomics core facilities at PBRC, which are supported in part by COBRE (NIH P20-RR021945) and CNRU (NIH 1P30-DK072476) center grants.

References

- Bamshad M, Aoki VT, Adkison MG, Warren WS, Bartness TJ. Central nervous system origins of the sympathetic nervous system outflow to white adipose tissue. *Am J Physiol.* 1998; 275:R291–R299. [PubMed: 9688991]
- Bamshad M, Song CK, Bartness TJ. CNS origins of the sympathetic nervous system outflow to brown adipose tissue. *Am J Physiol.* 1999; 276:R1569–R1578. [PubMed: 10362733]

- Banfield BW, Kaufman JD, Randall JA, Pickard GE. Development of pseudorabies virus strains expressing red fluorescent proteins: new tools for multisynaptic labeling applications. *J Virol.* 2003; 77:10106–10112. [PubMed: 12941921]
- Berthoud HR, Patterson LM, Sutton GM, Morrison C, Zheng H. Orexin inputs to caudal raphe neurons involved in thermal, cardiovascular, and gastrointestinal regulation. *Histochem Cell Biol.* 2005; 123:147–156. [PubMed: 15742197]
- Berthoud HR, Morrison C. The brain, appetite, and obesity. *Annu Rev Psychol.* 2008; 59:55–92. [PubMed: 18154499]
- Coleman DL. Increased metabolic efficiency in obese mutant mice. *Int J Obes.* 1985; 9 Suppl 2:69–73. [PubMed: 4066138]
- Collins S, Kuhn CM, Petro AE, Swick AG, Chrnyk BA, Surwit RS. Role of leptin in fat regulation. *Nature.* 1996; 380:677. [PubMed: 8614460]
- Commings SP, Watson PM, Levin N, Beiler RJ, Gettys TW. Central leptin regulates the UCP1 and ob genes in brown and white adipose tissue via different beta-adrenoceptor subtypes. *J Biol Chem.* 2000; 275:33059–33067. [PubMed: 10938091]
- Dunbar JC, Hu Y, Lu H. Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. *Diabetes.* 1997; 46:2040–2043. [PubMed: 9392493]
- Elias CF, Lee C, Kelly J, Aschkenasi C, Ahima RS, Couceyro PR, Kuhar MJ, Saper CB, Elmquist JK. Leptin activates hypothalamic CART neurons projecting to the spinal cord. *Neuron.* 1998; 21:1375–1385. [PubMed: 9883730]
- German J, Kim F, Schwartz GJ, Havel PJ, Rhodes CJ, Schwartz MW, Morton GJ. Hypothalamic leptin signaling regulates hepatic insulin sensitivity via a neurocircuit involving the vagus nerve. *Endocrinology.* 2009; 150:4502–4511. [PubMed: 19574396]
- Grill HJ, Schwartz MW, Kaplan JM, Foxhall JS, Breininger J, Baskin DG. Evidence that the caudal brainstem is a target for the inhibitory effect of leptin on food intake. *Endocrinology.* 2002; 143:239–246. [PubMed: 11751615]
- Grill HJ, Hayes MR. The nucleus tractus solitarius: a portal for visceral afferent signal processing, energy status assessment and integration of their combined effects on food intake. *Int J Obes (Lond).* 2009; 33 Suppl 1:S11–S15. [PubMed: 19363500]
- Haque MS, Minokoshi Y, Hamai M, Iwai M, Horiuchi M, Shimazu T. Role of the sympathetic nervous system and insulin in enhancing glucose uptake in peripheral tissues after intrahypothalamic injection of leptin in rats. *Diabetes.* 1999; 48:1706–1712. [PubMed: 10480598]
- Haynes WG, Sivitz WI, Morgan DA, Walsh SA, Mark AL. Sympathetic and cardiorenal actions of leptin. *Hypertension.* 1997; 30:619–623. [PubMed: 9322991]
- Kamohara S, Burcelin R, Halaas JL, Friedman JM, Charron MJ. Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature.* 1997; 389:374–377. [PubMed: 9311777]
- Kerman IA, Emanuel BA, Yates BJ. Vestibular stimulation leads to distinct hemodynamic patterning. *Am J Physiol Regul Integr Comp Physiol.* 2000; 279:R118–R125. [PubMed: 10896872]
- Kerman IA, Enquist LW, Watson SJ, Yates BJ. Brainstem substrates of sympatho-motor circuitry identified using trans-synaptic tracing with pseudorabies virus recombinants. *J Neurosci.* 2003; 23:4657–4666. [PubMed: 12805305]
- Kerman IA, Akil H, Watson SJ. Rostral elements of sympatho-motor circuitry: a virally mediated transsynaptic tracing study. *J Neurosci.* 2006; 26:3423–3433. [PubMed: 16571749]
- Kerman IA, Bernard R, Rosenthal D, Beals J, Akil H, Watson SJ. Distinct populations of presympathetic-premotor neurons express orexin or melanin-concentrating hormone in the rat lateral hypothalamus. *J Comp Neurol.* 2007; 505:586–601. [PubMed: 17924541]
- Lee TK, Lois JH, Troupe JH, Wilson TD, Yates BJ. Transneuronal tracing of neural pathways that regulate hindlimb muscle blood flow. *Am J Physiol Regul Integr Comp Physiol.* 2007; 292:R1532–R1541. [PubMed: 17158263]
- Lee Y, Wang MY, Kakuma T, Wang ZW, Babcock E, McCorkle K, Higa M, Zhou YT, Unger RH. Liporegulation in diet-induced obesity. The antisteatotic role of hyperleptinemia. *J Biol Chem.* 2001; 276:5629–5635. [PubMed: 11096093]
- Leininger GM, Jo YH, Leshan RL, Louis GW, Yang H, Barrera JG, Wilson H, Opland DM, Faouzi MA, Gong Y, Jones JC, Rhodes CJ, Chua S Jr, Diano S, Horvath TL, Seeley RJ, Becker JB,

- Munzberg H, Myers MG Jr. Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. *Cell Metab.* 2009; 10:89–98. [PubMed: 19656487]
- Leshan RL, Bjornholm M, Munzberg H, Myers MG Jr. Leptin receptor signaling and action in the central nervous system. *Obesity (Silver Spring).* 2006; 14 Suppl 5:208S–212S. [PubMed: 17021368]
- Leshan RL, Louis GW, Jo YH, Rhodes CJ, Munzberg H, Myers MG Jr. Direct innervation of GnRH neurons by metabolic- and sexual odorant-sensing leptin receptor neurons in the hypothalamic ventral premammillary nucleus. *J Neurosci.* 2009; 29:3138–3147. [PubMed: 19279251]
- Levin N, Nelson C, Gurney A, Vandlen R, de Sauvage F. Decreased food intake does not completely account for adiposity reduction after ob protein infusion. *Proc Natl Acad Sci U S A.* 1996; 93:1726–1730. [PubMed: 8643697]
- McAllen RM, Dampney RA. Vasomotor neurons in the rostral ventrolateral medulla are organized topographically with respect to type of vascular bed but not body region. *Neurosci Lett.* 1990; 110:91–96. [PubMed: 1970144]
- Minokoshi Y, Haque MS, Shimazu T. Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. *Diabetes.* 1999; 48:287–291. [PubMed: 10334303]
- Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, Kahn BB. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature.* 2002; 415:339–343. [PubMed: 11797013]
- Minokoshi Y, Kahn BB. Role of AMP-activated protein kinase in leptin-induced fatty acid oxidation in muscle. *Biochem Soc Trans.* 2003; 31:196–201. [PubMed: 12546684]
- Montanaro MS, Allen AM, Oldfield BJ. Structural and functional evidence supporting a role for leptin in central neural pathways influencing blood pressure in rats. *Exp Physiol.* 2005; 90:689–696. [PubMed: 16105939]
- Morgan DA, Thedens DR, Weiss R, Rahmouni K. Mechanisms mediating renal sympathetic activation to leptin in obesity. *Am J Physiol Regul Integr Comp Physiol.* 2008; 295:R1730–R1736. [PubMed: 18815209]
- Morrison SF. Activation of 5-HT1A receptors in raphe pallidus inhibits leptin-evoked increases in brown adipose tissue thermogenesis. *Am J Physiol Regul Integr Comp Physiol.* 2004; 286:R832–R837. [PubMed: 14742306]
- Morton GJ, Gelling RW, Niswender KD, Morrison CD, Rhodes CJ, Schwartz MW. Leptin regulates insulin sensitivity via phosphatidylinositol-3-OH kinase signaling in mediobasal hypothalamic neurons. *Cell Metab.* 2005; 2:411–420. [PubMed: 16330326]
- Myers MG Jr, Munzberg H, Leininger GM, Leshan RL. The geometry of leptin action in the brain: more complicated than a simple ARC. *Cell Metab.* 2009; 9:117–123. [PubMed: 19187770]
- Nogueiras R, Wiedmer P, Perez-Tilve D, Veyrat-Durebex C, Keogh JM, Sutton GM, Pfluger PT, Castaneda TR, Neschen S, Hofmann SM, Howles PN, Morgan DA, Benoit SC, Szanto I, Schrott B, Schurmann A, Joost HG, Hammond C, Hui DY, Woods SC, Rahmouni K, Butler AA, Farooqi IS, O'Rahilly S, Rohner-Jeanraud F, Tschop MH. The central melanocortin system directly controls peripheral lipid metabolism. *J Clin Invest.* 2007; 117:3475–3488. [PubMed: 17885689]
- Nonogaki K. New insights into sympathetic regulation of glucose and fat metabolism. *Diabetologia.* 2000; 43:533–549. [PubMed: 10855527]
- Oldfield BJ, Giles ME, Watson A, Anderson C, Colvill LM, McKinley MJ. The neurochemical characterisation of hypothalamic pathways projecting polysynaptically to brown adipose tissue in the rat. *Neuroscience.* 2002; 110:515–526. [PubMed: 11906790]
- Paxinos, G.; Franklin, KBJ. *The Mouse Brain in Stereotaxic Coordinates.* Vol. Vol.. Academic Press; 2001.
- Rahmouni K, Haynes WG, Mark AL. Cardiovascular and sympathetic effects of leptin. *Curr Hypertens Rep.* 2002; 4:119–125. [PubMed: 11884267]
- Rahmouni K, Morgan DA. Hypothalamic arcuate nucleus mediates the sympathetic and arterial pressure responses to leptin. *Hypertension.* 2007; 49:647–652. [PubMed: 17190874]

- Ray N, Enquist LW. Transcriptional response of a common permissive cell type to infection by two diverse alphaherpesviruses. *J Virol.* 2004; 78:3489–3501. [PubMed: 15016872]
- Scarpace PJ, Matheny M. Leptin induction of UCP1 gene expression is dependent on sympathetic innervation. *Am J Physiol.* 1998; 275:E259–E264. [PubMed: 9688627]
- Scott MM, Lachey JL, Sternson SM, Lee CE, Elias CF, Friedman JM, Elmquist JK. Leptin targets in the mouse brain. *J Comp Neurol.* 2009; 514:518–532. [PubMed: 19350671]
- Song CK, Enquist LW, Bartness TJ. New developments in tracing neural circuits with herpesviruses. *Virus Res.* 2005; 111:235–249. [PubMed: 15893400]
- Ukropec J, Anunciado RV, Ravussin Y, Kozak LP. Leptin is required for uncoupling protein-1-independent thermogenesis during cold stress. *Endocrinology.* 2006; 147:2468–2480. [PubMed: 16469807]
- Wijers SL, Schrauwen P, Saris WH, van Marken Lichtenbelt WD. Human skeletal muscle mitochondrial uncoupling is associated with cold induced adaptive thermogenesis. *PLoS One.* 2008; 3:e1777. [PubMed: 18335051]
- Zheng H, Patterson LM, Morrison C, Banfield BW, Randall JA, Browning KN, Travagli RA, Berthoud HR. Melanin concentrating hormone innervation of caudal brainstem areas involved in gastrointestinal functions and energy balance. *Neuroscience.* 2005; 135:611–625. [PubMed: 16111819]

Figure 1. Muscle-derived PRV labeling of the brainstem

mRFP-expressing PRV was injected into the left gastrocnemius muscle, and mice perfused 5.5 days after injection. The current figure provides a schematic illustration of the distribution of PRV within a series of coronal sections of the brainstem, with each dot representing one PRV-positive neuron. Numbers on the left of each section represent the position of that section relative to bregma. The calibration mark = 1 mm.

Figure 2. Colocalization of leptin receptor and muscle derived PRV in the NTS

Schematic illustration of colocalization of leptin receptor and muscle-derived PRV expression within a series of coronal sections of the mouse NTS following PRV injection into the left gastrocnemius muscle. PRV-infected neurons are illustrated as filled circles, while LepRb/GFP-positive neurons are illustrated as open circles. Double labeled neurons are illustrated as triangles. Each shape represents a single neuronal. Numbers on the left of each section represent the position of that section relative to the bregma. The calibration mark = 500 μ m.

Figure 3. Quantification of leptin receptor and PRV colocalization in the NTS

A. Total neuronal counts of PRV, LepRb/GFP, or double labeled neurons from every third section throughout the NTS. **B.** Percentage of double-labeled neurons within the total population of PRV and LepRb positive neurons (%PRV = percentage of PRV positive neurons that also contained leptin receptor; %LepR = percentage of LepRb positive neurons that also contained PRV). **C.** Confocal photomicrographs illustrating PRV and LepRb colocalization within the NTS. The lower power panels reflect a higher-power view of the inset section. The calibration mark = 100 μ m for top panels and 25 μ m for lower panels.

Figure 4. Muscle-derived PRV labeling of the hypothalamus

mRFP-expressing PRV was injected into the left gastrocnemius muscle, and mice perfused 5.5 days after injection. The current figure provides a schematic illustration of the distribution of PRV within a series of coronal sections through the hypothalamus, with each dot representing one PRV-positive neuron. Numbers on the left of each section represent the position of that section relative to bregma. The calibration mark = 1 mm.

Figure 5. Distribution of leptin receptor and PRV neurons within the hypothalamus

A. Total neuronal counts of PRV-positive and LepRb/GFP-positive neurons throughout various hypothalamic nuclei. Neuronal counts were assessed on every sixth hypothalamic section. **B.** Representative confocal photomicrographs within the PVH and LH, illustrating the absence of colocalization within these brain areas. Calibration mark = 50 μ M.

Figure 6. Leptin receptor and muscle-derived PRV colocalization in the RCH

Schematic illustration of colocalization of leptin receptor and muscle derived PRV expression within a series of coronal sections of the mouse RCH following PRV injection into the left gastrocnemius muscle. PRV-infected neurons are illustrated as filled circles, while LepRb/GFP-positive neurons are illustrated as open circles. Double labeled neurons are illustrated as triangles. Each shape represents a single neuronal. Numbers on the left of each section represent the position of that section relative to the bregma. The calibration mark = 500 μ m.

Figure 7. Quantification of leptin receptor and PRV colocalization in the RCH

A. Total neuronal counts of PRV, LepRb/GFP, or double labeled neurons from every third section throughout the RCH. **B.** Percentage of double-labeled neurons within the total population of PRV and LepRb positive neurons (%PRV = percentage of PRV positive neurons that also contained leptin receptor; %LepR = percentage of LepRb positive neurons that also contained PRV). **C.** Confocal photomicrographs illustrating PRV and LepRb colocalization within the RCH. The lower power panels reflect a higher-power view of the inset section. The calibration mark = 250 μ m for top panels and 50 μ m for lower panels.