Abstract

Hypothalamic leptin action promotes negative energy balance and modulates glucose homeostasis, as well as serving as a permissive signal to the neuroendocrine axes that control growth and reproduction. Since the initial discovery of leptin 20 years ago, we have learned a great deal about the molecular mechanisms of leptin action. An important aspect of this has been the dissection of the cellular mechanisms of leptin signaling, and how specific leptin signals influence physiology. Leptin acts via the long form of the leptin receptor LepRb. LepRb activation and subsequent tyrosine phosphorylation recruits and activates multiple signaling pathways, including STAT transcription factors, SHP2 and ERK signaling, the IRS-protein/PI3Kinase pathway, and SH2B1. Each of these pathways controls specific aspects of leptin action and physiology. Important inhibitory pathways mediated by suppressor of cytokine signaling proteins and protein tyrosine phosphatases also limit physiologic leptin action. This review summarizes the signaling pathways engaged by LepRb and their effects on energy balance, glucose homeostasis, and reproduction. Particular emphasis is given to the multiple mouse models that have been used to elucidate these functions in vivo.

Key Words
- leptin
- signal transduction
- obesity
- hypothalamus

Introduction

Obesity and its many comorbidities present a significant challenge to public health in the USA. The healthcare costs associated with obesity totaled more than $147 billion annually. In addition to the economic burden, obesity results in premature death and disability from stroke, cardiovascular disease, and type 2 diabetes mellitus (http://www.cdc.gov/obesity/data/adult.html accessed 6/29/14). Furthermore, the obesity epidemic is no longer confined to the USA. Worldwide, more than 1.4 billion adults were overweight or obese in 2008 (Danaei et al. 2011). Clearly, the need for anti-obesity therapies is large and growing larger, yet no pharmacotherapies have been achieved more than minimal success in promoting long-term weight loss.

At its most basic level, body weight is determined by the amount of energy taken in relative to energy expenditure (Schwartz et al. 2000). If energy intake exceeds energy expenditure, excess energy accumulates in the form of triglycerides stored in adipose tissue, resulting in weight gain and obesity. However, the brain integrates signals of long-term energy stores with other physiologic inputs to modulate energy intake relative to energy expenditure. When adipose energy (fat) stores fall, hunger increases and energy expenditure decreases to defend body energy stores; conversely, the brain responds to nutritional surfeit by permitting increased energy expenditure and decreased feeding to maintain a constant body weight.
One of the most important and widely studied players in the control of energy balance is the hormone leptin (Friedman & Halaas 1998, Elmquist et al. 2005). Leptin was discovered by Zhang et al. (1994). Defects in leptin production underlie the massive obesity observed in ob/ob mice. Leptin is produced in adipose tissue in proportion to triglyceride stores, and serves as a critical indicator of an organism’s long-term energy status (Frederich et al. 1995a, Maffei et al. 1995). Leptin acts primarily in the brain, especially the hypothalamus, where its action is integrated with that of other adipokines, gastrokines, and other signals to coordinate energy homeostasis (Friedman & Halaas 1998, Bates & Myers 2003, Myers et al. 2009, Ring & Zeltser 2010). In addition to leptin-deficient ob/ob mice, rare human mutations resulting in leptin deficiency have also been identified; leptin-deficient mice and humans display hyperphagia, decreased energy expenditure, and early-onset obesity (Montague et al. 1997, Farooqi et al. 1999). Leptin receptor (LepRb)-deficient humans and db/db mice display a similar phenotype (Tartaglia et al. 1995, Chua et al. 1996). Numerous studies have elaborated the critical role of leptin in the modulation of energy balance: the lack of leptin, as in starvation or genetic leptin deficiency, increases hunger while promoting an energy-sparing program of neuroendocrine and autonomic changes, including decreased sympathetic nervous system tone, thyroid function, growth, and reproduction (Ahima et al. 1997). Leptin treatment largely reverses these changes (Farooqi et al. 1999, 2002). Decreased leptin also promotes a variety of other behavioral and physiologic changes to respond appropriately to low energy stores (Lu et al. 2006, Liu et al. 2010, 2011).

Despite the initial heralding of leptin as a potential cure for human obesity, most obese humans exhibit high circulating leptin concentrations (Maffei et al. 1995). Serum leptin increases in proportion to body fat percentage; obese patients secrete leptin at levels appropriate for their increased adipose mass and display elevated leptin concentrations (‘hyperleptinemia’) relative to lean controls (Tobe et al. 1999). Clearly, however, these high circulating leptin levels do not suffice to restore body adiposity to lean levels, as might be predicted based on the sensitivity of organisms to decreases in leptin signaling. Whether this inability of leptin to suppress feeding in the face of obesity results from an intrinsic or acquired defect in leptin action, or rather simply reflects the inability of homeostatic controls to overcome hedonic feeding drives remains a matter of debate. This controversy serves to underscore the importance of developing a more complete understanding of leptin signaling, its cellular effects, target neural pathways, and integration with other determinants of energy homeostasis (Figs 1 and 2).

**Figure 1**
Leptin signaling and biological function. Leptin binds to LepRb, activating the associated JAK2 tyrosine kinase. Activated JAK2 phosphorylates the intracellular tail of LepRb on three tyrosine residues. Phosphorylated Tyr985 recruits SHP2, which participates in ERK signaling; Tyr985 also serves as a binding site for the negative feedback regulator, SOCS3. Phosphorylated Tyr1077, partially mediates leptin’s control of reproduction; while STAT5 binds this site, STAT5 does not appear to participate in this effect of leptin. Phosphorylated Tyr1138 engages the STAT3 transcription factor. LepRb → STAT3 signaling represents the primary mechanism by which leptin regulates energy balance, although the target genes of STAT3 in LepRb neurons remain undiscovered. Leptin also recruits the IRS2 → PI3K and SH2B1 pathways, although the mechanism of their recruitment to LepRb remains unclear. Some glucoregulatory and reproductive actions of LepRb appear to be mediated by unknown signals that function independently of LepRb tyrosine phosphorylation sites.
Leptin and the LepRb

Leptin is a 146 amino acid protein produced in white adipose tissue in proportion to triglyceride stores (Frederich et al. 1995b). Once secreted into the circulation, leptin travels to the brain, where it enters the CNS, presumably via the choroid plexus and circumventricular organs. In the brain, leptin acts by binding and activating the long form of LepRb, which is expressed primarily on specialized subsets of neurons in certain hypothalamic and brainstem nuclei (Tartaglia 1997, Elias et al. 2000, Scott et al. 2009, Patterson et al. 2011). Mutations that inactivate LepRb, as well as antagonists of LepRb activation, confirm that leptin binding to LepRb is required for its biological activity (Chen et al. 1996, Shpilman et al. 2011). While the LEPR gene encodes multiple isoforms (LepRa-f in rats), only LepRb contains the full intracellular domain necessary for the activation of critical second messenger pathways and normal leptin action (Chua et al. 1996, 1997, Lee et al. 1996, Tartaglia 1997). Many functions for the other ‘short’ forms of the receptor have been hypothesized, including actions as a serum-binding protein that functions in leptin stabilization or sequestration (Zastrow et al. 2003, Yang et al. 2004, Zhang & Scarpone 2009), or as a leptin transporter (Bjorbaek et al. 1998a, Kastin et al. 1999), but LepRb alone suffices for the control of energy balance, glucose homeostasis, and other leptin effects, and LepRb thus constitutes the focus of this review.

Peripheral actions of leptin

Multiple studies have attempted to assess the role of leptin in the periphery. Mice with ablated hepatic leptin signaling had normal body weight and blood glucose levels, but were protected from high-fat diet or age-induced insulin intolerance. Mice in which LepRb was deleted from the pancreas using a Pdxcre or Ripcre also showed improvements in glucose tolerance (Morioka et al. 2007, Huynh et al. 2010). However, interpretation of these results is confounded by hypothalamic CRE expression in both the PDx and RIP models (Schwartz et al. 2010, Wicksteed et al. 2010). LepRb expression has also been demonstrated in perivascular intestinal cells, although the function of LepRb in these cells has not been determined.
Central actions of leptin

Within the brain, leptin acts on multiple populations of LepRb neurons – primarily in the hypothalamus and brainstem (Scott et al. 2009, Patterson et al. 2011). While leptin action in the nucleus of the solitary tract plays a role in the modulation of satiety, and ventral tegmental area LepRb contributes to the control of reward and aversion, hypothalamic LepRb appears to mediate the lion’s share of leptin action on energy balance (Hommel et al. 2006, Hayes et al. 2010, Ring & Zeltser 2010). Within the hypothalamus, leptin acts on multiple populations of LepRb-expressing neurons, including those in the lateral hypothalamic area and the ventromedial, dorsomedial, ventral premammillary, and arcuate (ARC) nuclei (Scott et al. 2009, Patterson et al. 2011). Each of these sites contains multiple distinct types of LepRb cells, each of which contributes uniquely to leptin action. The most studied site of leptin action is the ARC, where leptin inhibits orexigenic agouti-related protein/neuropeptide Y-containing (AgRP/NPY) neurons and stimulates anorexigenic proopiomelanocortin (POMC)-containing neurons. POMC neurons produce anorexigenic neuropeptides, while AgRP is a potent antagonist of the melanocortin system and NPY mediates additional orexigenic signals (Schwartz et al. 2000).

LepRb signaling

LepRb is an IL6-type class I cytokine receptor, consisting of an extracellular leptin-binding domain, a single-pass membrane spanning domain, and an intracellular tail that contains binding domains for multiple signaling proteins (Tartaglia et al. 1995, Baumann et al. 1996). LepRb is present on the cell membrane as a mixture of monomers and dimers (Devos et al. 1997). Unlike many other cytokine receptors, ligand binding does not appear to activate LepRb by promoting receptor dimerization, but rather promotes a conformational change that results in the autophosphorylation and activation of JAK2, which is constitutively bound to Box1 and Box2 motifs in the membrane-proximal portion of LepRb (Banks et al. 2000, Kloek et al. 2002). Activated JAK2 phosphorylates LepRb on three tyrosine residues in mice: Tyr985, Tyr1077, and Tyr1138 (Banks et al. 2000, Gong et al. 2007). Each of these phosphorylated tyrosine (pY) residues represents a Src homology 2 (SH2)-binding motif that recruits specific SH2-containing effector proteins to the receptor to mediate subsequent signaling.

Leptin binding to LepRb results in the activation of several major signaling pathways. Importantly, phosphorylation of Tyr1138 results in the recruitment of STAT3 to LepRb, to permit its phosphorylation (pSTAT3) and activation by JAK2 (White et al. 1997, Banks et al. 2000). Activated pSTAT3 translocates to the nucleus, where it mediates changes in the expression of target genes, including suppressor of cytokine signaling 3 (Socs3) (which encodes a feedback inhibitor of LepRb signaling) (Bjorbaek et al. 1999). Phosphorylation of Tyr985 recruits protein tyrosine phosphatase 2 (SHP2; PTPN1) to LepRb, contributing to the activation of the ERK signaling pathway (Banks et al. 2000, Bjorbaek et al. 2001). Tyr985 also serves as the binding site for SOCS3 and thus plays a prominent role in the feedback inhibition of LepRb (Bjorbaek et al. 2000). Phosphorylated Tyr1077 promotes the recruitment and activation of STAT5; Tyr1138 may also contribute to STAT5 activation (Gong et al. 2007).

Another SH2 domain protein, SH2B1, also participates in LepRb signaling. In addition to increasing the amplitude of LepRb signaling via JAK2, SH2B1 may control specific downstream LepRb signals, including insulin receptor substrate (IRS)-proteins (Duan et al. 2004, Ren et al. 2005). IRS-proteins also participate in leptin action; they control the phosphatidylinositol 3-kinase (PI3K) pathway, and the subsequent regulation of Akt→FoxO1 and mTORC1 signaling (Niswender et al. 2001, Kim et al. 2006, Kitamura et al. 2006). The mechanism(s) whereby LepRb modulates this pathway remains obscure; some data suggest a potential role for poorly understood LepRb signaling that occurs independently of LepRb pY sites.

Leptin signaling and physiology

LepRb → STAT3 signaling

Multiple LepRb signaling pathways coordinate the regulation of energy homeostasis. Of these, the Ty1138→ pSTAT3 pathway plays an especially prominent role (Bates & Myers 2003). Mice containing a substitution mutation of LepRb Ty1138 (which renders LepRb incapable of recruiting and activating STAT3; s/s mice) display
hyperphagia and obesity approaching that of db/db animals (although linear growth, fertility, and glucose homeostasis are relatively protected in s/s relative to db/db mice) (Bates et al. 2003, 2004, 2005). Furthermore, brain-specific STAT3-knockout mice (STAT3N/-) exhibit severe obesity (Gao et al. 2004). Mice in which STAT3 was deleted specifically in LepRb neurons (LepRbSTAT3-KO) similarly develop hyperphagic obesity with some preservation of glucose homeostasis (Piper et al. 2007). These studies highlight the importance of LepRb Tyr1138 → STAT3 signaling for the regulation of body weight, but suggest some regulation of growth, reproduction, and glucose homeostasis by leptin independently of this pathway.

The role of STAT3 signaling in energy balance in discrete neural populations has been best characterized in the ARC. As might be expected, specific deletion of STAT3 from AgRP neurons results in moderate obesity, increased Npy expression, and decreased sensitivity to leptin (Gong et al. 2008). STAT3 deletion from POMC neurons also increases adiposity, but the effect is milder than that observed for the AgRP-specific knockout, suggesting a greater role for STAT3 in leptin action in AgRP neurons than in POMC cells (Xu et al. 2007). In contrast to STAT3 deletion studies, the interpretation of studies in which a mutant, transcriptionally active, form of STAT3 (STAT3-C) is expressed in ARC neurons is more complicated. While STAT3-C expression in AgRP neurons promotes leanness, STAT3-C expression in POMC neurons results in obesity (Mesaros et al. 2008, Ernst et al. 2009). Agp expression is not altered in AgrpSTAT3-C mice, consistent with the notion that Agp expression is more sensitive to modulation by PI3K than by STAT3 (see below) (Mesaros et al. 2008). It is possible that the mild obesity resulting from STAT3-C expression in POMC neurons results from altered transcriptional activity of this isoform relative to native STAT3, but STAT3-C also promotes Socs3 expression, which could limit endogenous leptin action despite increased transcription mediated by STAT3-C. Interestingly, although the Pomc promoter contains known STAT3-binding sites (Munzberg et al. 2003) and Pomc expression is decreased in s/s mice and animals with neuronal STAT3 ablation (Bates et al. 2003, Gao et al. 2004), Pomc expression is decreased in PomcSTAT3-C animals (Ernst et al. 2009), suggesting that while Socs3 represents a direct STAT3 target, the control of ARC Pomc expression may reflect the effects of additional and/or downstream LepRb signals, as well. Additionally, none of the phenotypes resulting from the modulation of LepRb→STAT3 signaling in POMC or AgRP neurons approach that of brain or hypothalamus-wide modulation, suggesting that LepRb→STAT3 signaling in other, non-ARC LepRb cells contributes to the control of energy balance during LepRb→STAT3 signaling.

**Tyr985-dependent signaling, SOCS3, and SHP2**

In contrast to the obese phenotype that results from disruption of LepRb→STAT3 signaling, mice with a mutation in Tyr985 display a lean phenotype (which is especially pronounced in females). These mice also display decreased hypothalamic Agrp expression, increased pSTAT3, exaggerated sensitivity to exogenous leptin, and resistance to DIO (Bjornholm et al. 2007). These results are consistent with increased LepRb signaling due to decreased LepRb feedback inhibition via disruption of SOCS3 binding. Indeed, as for mice mutant for LepRb Tyr985, disruption of Socs3 in the brain decreases adiposity (more dramatically in female than in male mice) and increases the response to exogenous leptin (Mori et al. 2004).

In addition to its role in feedback inhibition, Tyr985 may also coordinate energy homeostasis via SHP2/ERK signaling (Bjorbaek et al. 2000, 2001). As a tyrosine phosphatase, SHP2 was initially investigated as a potential negative regulator of leptin signaling. However, deletion of Shp2 from the forebrain disrupts ERK signaling and promotes early-onset obesity (Zhang et al. 2004). Furthermore, deletion of Shp2 from POMC neurons results in mild obesity and increased susceptibility to DIO (Banno et al. 2010). Similarly, female mice expressing a dominant active SHP2 mutant in the brain are resistant to DIO (He et al. 2012). Thus, these data are consistent with the notion that LepRb→SHP2 signaling is important for leptin action and the control of energy homeostasis, rather than SHP2-mediated feedback inhibition on LepRb. While SHP2 plays an essential role in the control of energy homeostasis, however, the promiscuity of SHP2 (which plays roles in many signaling pathways) renders it difficult to assess the specificity of SHP2 effects for LepRb signaling.

**Tyr1077 and STAT5**

LepRb→STAT5 signaling appears to have little impact on energy balance. While brain-wide STAT5 knockout mice develop late-onset obesity, this phenotype is quite mild (Lee et al. 2008). LepRb Tyr1077 mutants develop only mildly increased food intake and adiposity (Patterson et al. 2012). Furthermore, a recent study deleting STAT5 specifically in LepRb neurons has revealed no body weight phenotype; deleting both STAT3 and STAT5 that did not produce a more robust phenotype than deleting STAT3 alone (Singireddy et al. 2013). Also, Tyr1077 mutants enter
puberty normally, but have a prolonged inter-estrus interval, suggesting mild subfertility in these animals. However, LepR^{STATS-KO} animals display normal estrus cycling and fertility. Altogether, these studies suggest that Tyr_{1077} plays a minor role in the control of feeding and reproductive functions, but that STAT5 may not be the binding partner that mediates this effect.

**Other LepRb signals**

Although the tyrosine phosphorylation of LepRb is essential for the majority of leptin’s actions, mice in which Tyr_{985}, Tyr_{1077}, and Tyr_{1138} have all been replaced with phenylalanine (LepRb^{3F}) are less slightly less obese than db/db animals and display significant improvements in glucose homeostasis and fertility relative to db/db mice (Jiang et al. 2008). In contrast, mice express a LepRb truncation mutant (LepRb^{65}) that retains JAK2 signaling and activity but lacks Tyr_{985}, Tyr_{1077}, and Tyr_{1138} pheno-copy db/db animals and do not appear to be significantly protected from the obesity, diabetes and infertility that are hallmarks of impaired leptin signaling (Robertson et al. 2010). Thus, the improved phenotype seen in LepRb^{3F} mice relative to db/db animals does not result from JAK2 signaling alone, as the LepRb^{65} model reveals that JAK2 signaling is not sufficient to mediate these improvements. The differing phenotypes between mice expressing LepRb^{3F} and LepRb^{65} thus suggest the existence of non-canonical signaling pathway that may emanate from a distal site on LepRb, independently of LepRb pY sites. Further work will be required to identify this presumptive pathway.

While SH2B1 and IRS-protein/PI3K signaling contribute to leptin action, the mechanism(s) of their activation by LepRb remain somewhat unclear; no LepRb pY site has been definitively shown to mediate their recruitment. Thus, it is possible that one or both of these pathways constitute the presumptive LepRb pY-independent signaling pathway. Furthermore, these pathways may overlap, as SH2B1 recruits the IRS-protein/PI3K pathway during leptin signaling in cultured cells (Kim et al. 2000, Duan et al. 2004). However, the SH2B1 and IRS-protein/PI3K pathways contribute to energy balance in vivo. Sh2b1-null mice display severe early-onset obesity and hyperphagia (Ren et al. 2005). Furthermore, neuron-specific restoration of SH2B1 throughout the CNS rescues this phenotype, suggesting that CNS SH2B1 is crucial for the control of body weight (Morris et al. 2010). Unfortunately, the critical role of SH2B1 in insulin signaling (which is also significantly impacted by this deletion) as well as in signaling by other receptor tyrosine kinases renders it challenging to determine whether this phenotype results from only from the disruption of LepRb→SH2B1 signaling.

The roles for PI3K signaling in leptin action and the control of energy balance are also complicated. Leptin administration activates IRS-protein/PI3K signaling in the mediobasal hypothalamus, and ICV treatment with PI3K inhibitors inhibits leptin’s anorexigenic effects (Niswender et al. 2001), along with the ability of exogenous leptin to suppress Agrp mRNA expression in fasted rats (Morrison et al. 2005). Furthermore, deletion of IRS2 specifically in LepRb neurons results in obesity (although it does not impact the ability of LepRb to stimulate pSTAT3) (Sadagurski et al. 2012). Both in vitro and in vivo studies have also implicated PI3K signaling in the acute actions of leptin. Leptin treatment induces the depolarization of POMC neurons in slice recordings; these effects are abrogated by pretreatment with PI3K inhibitors (Hill et al. 2008). This effect is also perturbed in mice lacking the PI3K regulatory subunits p85α and p85β in POMC neurons (Hill et al. 2008). While these mice do not display gross phenotypic abnormalities, leptin’s ability to promote acute decreases in food intake is also disrupted. Studies in which the PI3K catalytic subunits p110α and p110β were deleted in AgRP or POMC neurons confirm these findings – mice lacking p110β in AgRP neurons are mildly lean, whereas mice lacking p110β in POMC neurons are more sensitive to DIO (Al-Qassab et al. 2009). It is unclear however, whether these results emanate from disrupted LepRb-PI3K signaling, or from alternations in IR-PI3K signaling, especially in light of data that suggests that leptin and insulin activate non-overlapping populations of POMC neurons (Williams et al. 2010). Together, these data suggest that leptin-induced PI3K signaling has a limited effect on energy balance. However, the importance of the LepRb-PI3K pathway for the glucoregulatory or reproductive functions of leptin is yet to be determined.

**Negative regulation of leptin signaling**

Multiple pathways and proteins inhibit LepRb. Given its role as an inhibitor of LepRb signaling, the mechanisms of action for SOCS3 have been a point of considerable interest. SOCS3 binds to LepRb Tyr_{985} and mediates negative feedback by directly inhibiting JAK2 activity and/or targeting the receptor-JAK2 complex for proteasomal degradation (Bjorbaek et al. 1998b, 1999, 2000). Neuron-wide deletion of Socs3 using either nestin-cre (Socs3^{−/−}) or synapsin-cre confers a significant resistance...
to diet-induced obesity (Mori et al. 2004). Soc3^{N−/−} mice also display increased leptin sensitivity as measured by both leptin-induced food intake and STAT3 phosphorylation, as well as by increased PI3K activity. While Soc3 has not been disrupted specifically in LepRb neurons, overexpression of Soc3 in LepRb neurons (LepRbSocs3-OE) yields an unexpected phenotype of slightly increased leanness (Reed et al. 2010). This may result from a compensatory increase in STAT3 at baseline and a corresponding increase in pSTAT3 levels after leptin treatment, although the mechanism for this is unclear and would seem to be a bit counter-intuitive. Clearly, however, the function of SOCS3 may not be as uniform or straightforward as initially thought.

Because high-fat diet induces Soc3 expression in the ARC, ARC populations have been posited to be a major site of leptin resistance. As a result, the role of Soc3 in arcuate POMC and AgRP neurons has been extensively studied. As with Soc3^{N−/−} mice, POMC^{Socs3-KO} mice are resistant to DIO, but display normal body weight on chow diet (Kievit et al. 2006). Interestingly, POMC^{Socs3-KO} mice also have improved glucose homeostasis on a chow diet, suggesting that POMC neurons may be a critical site of LepRb/SOC3 signaling in the control of peripheral blood glucose levels. Unlike LepRbSocs3-OE mice, mice overexpressing Soc3 in POMC neurons develop mild obesity on a chow diet, and acute leptin resistance (as assessed by leptin-induced inhibition of feeding) before any divergence in body weight (Reed et al. 2010). These animals also display a POMC neuron-restricted reduction in the pSTAT3 response to leptin, suggesting that potential compensatory mechanisms induced in the LepRbSocs3-OE model were not activated in this more restricted cell population. AgRP^{Socs3-OE} mice also display early-onset leptin resistance, and slightly abnormal glucose homeostasis, but no alterations in body weight (Olofsson et al. 2013). Thus, while decreasing Soc3 levels may prove protective against obesity, the modest body weight changes that occur with overexpression of Soc3 suggest that increased Soc3 levels may reflect hyperleptinemia and increased overall leptin signaling, rather than promoting obesity, per se.

Protein tyrosine phosphatases (PTPases) also modulate the amplitude and duration of LepRb signaling. Protein tyrosine phosphatase 1B (PTP1B) has been the most extensively studied of these, but other PTPs such as TCPPTP and RPTPz also play critical roles in both leptin and insulin signaling (see review by Tsou & Bence (2013)). PTP1B is a promiscuous phosphatase that attenuates signaling by the receptor for insulin as well as other receptors, in addition to LepRb. In vitro, PTP1B dose-dependently suppresses the leptin-stimulated phosphorylation of Jak2 and pSTAT3 (Zabolotny et al. 2002). In vivo, whole-body PTP1B knockout (PTP1B^{TKO}) results in a lean phenotype, resistance to DIO, and increased sensitivity to exogenous leptin, consistent with the interpretation that PTP1B is a negative regulator of LepRb signaling (Klaman et al. 2000). Interpretation of the PTP1B^{TKO} model is complicated by the promiscuity of PTP1B and its broad pattern of expression, however, provoking more focused studies of the sites and mechanisms of its action. Pan-neuronal deletion of PTP1B also induces a lean phenotype, whereas liver or muscle-specific deletion has no effect, and adipose-specific deletion actually causes weight gain (perhaps due to enhanced adipose insulin signaling) (Bence et al. 2006). LepRb neuron-specific PTP1B deletion (LepRb^{PTP1B-KO}) results in a leaner phenotype than that observed in the PTP1B^{TKO} mice, suggesting that this model may have unmasked an even more important role for PTP1B in LepRb neurons that may have been opposed by other tissue (e.g., adipose) effects in the PTP1B^{TKO} model (Tsou et al. 2012). The specificity of PTP1B action on LepRb for the development of the lean phenotype is supported by the similar phenotypes of hypothalamic LepRb knockout and LepRb/PTP1B double-knockout mice, suggesting the LepRb dependence of the lean phenotype of PTP1B-null animals (Tsou et al. 2014). Interestingly, heterozygous LepRb^{PTP1B+/−} mice display as strong a phenotype as LepRb^{PTP1B-KO}, underscoring the importance of appropriate levels of phosphatase action in the control of LepRb signaling (Tsou et al. 2012).

**Future directions: leptin signaling and gene transcription**

Despite the early identification of LepRb→STAT3 signaling as the primary mechanism for leptin’s control of energy balance, LepRb→STAT3 target genes remain poorly defined. Currently, the list of genes known to be regulated by leptin in vivo is short: Soc3, Pomp, Cart (Cartpt), Agrp, and Npy. LepRb→STAT3 signaling is required for appropriate Soc3, Pomp, and Agrp gene expression, although (as noted above) Pomp and Agrp may represent indirect targets of STAT3 and/or may be partly controlled by other pathways; PI3K appears to play a role in the control of Agrp and Npy expression. Furthermore, of these five genes, only Soc3 is thought to be induced in multiple LepRb populations; Pomp, Agrp, Npy, and Cart expression are restricted to circumscribed populations and do not contribute to leptin action in
the majority of LepRb neurons. This dearth of information about LepRb→STAT3 target genes can largely be attributed to the challenge of specifically isolating LepRb neurons from the hypothalamic milieu; LepRb neurons comprise approximately <5% of all hypothalamic neurons, making it challenging to identify cell-autonomous changes in gene transcription for any subset of neurons. Clearly, more work will be necessary to identify the hypothalamic gene targets of LepRb and STAT3 signaling. These transcripts will be responsible for much of leptin action and may represent potential targets for therapy, in addition to shedding light on the mechanisms of leptin action.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Funding

Supported by the Michigan Diabetes Research Center (NIH Grant P30 DK020572) the NIH (DK056731, DK78056, DK098853), the American Diabetes Association, and the Marilyn H Vincent Foundation to M G M, NIH DK097861 to M B A.

References


Elmqquist JK, Coppit R, Balthasar N, Ichinose M & Lowell BB 2005 Identifying hypothalamic pathways controlling food intake,


DOI: 10.1530/JOE-14-0404, http://joe.endocrinology-journals.org

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Received in final form 5 August 2014
Accepted 8 August 2014