



Leptin signalling pathways in hypothalamic neurons

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Abstract Leptin is the most critical hormone in the homeostatic regulation of energy balance among those so far discovered. Leptin primarily acts on the neurons of the mediobasal part of hypothalamus to regulate food intake, thermogenesis, and the blood glucose level. In the hypothalamic neurons, leptin binding to the long form leptin receptors on the plasma membrane initiates multiple signaling cascades. The signaling pathways known to mediate the actions of leptin include JAK–STAT signaling, PI3K–Akt–FoxO1 signaling, SHP2–ERK signaling, AMPK signaling, and mTOR–S6K signaling. Recent evidence suggests that leptin signaling in hypothalamic neurons is also linked to primary cilia function. On the other hand, signaling molecules/pathways mitigating leptin actions in hypothalamic neurons have been extensively investigated in an effort to treat leptin resistance observed in obesity. These include SOCS3, tyrosine phosphatase PTP1B, and inflammatory signaling pathways such as IKK–NFκB and JNK signaling, and ER stress–mitochondrial signaling. In this review, we discuss leptin signaling

pathways in the hypothalamus, with a particular focus on the most recently discovered pathways.

Keywords Leptin · Signaling · Hypothalamus · Neurons · Obesity · Cilia

Introduction

The regulation of food intake and energy expenditure by the central nervous system (CNS) is critical for maintaining whole body energy homeostasis. Among the brain regions, the hypothalamus plays a central role in the control of energy balance. Neurons in the hypothalamic arcuate nucleus (ARC) monitor the body energy state by sensing the blood levels of key metabolic hormones and nutrients. Leptin and leptin receptor signaling are most crucial of the factors identified to date that coordinate the control of food intake and energy expenditure in response to an altered energy state [1]. Leptin primarily acts on the ARC neurons producing anorexigenic proopiomelanocortin (POMC) and orexigenic neuropeptide Y (NPY)/*Agouti*-related protein (AgRP). Leptin suppresses energy intake and stimulates energy expenditure, leading to a reduction in stored body fat.

In 1950, the first obese mouse (*ob/ob*, now refer to *Lep^{ob}*) was reported by the Jackson Laboratory [2]. This mouse showed marked obesity with increased fat mass, massive hyperphagia and mild symptoms of diabetes. After 16 years, Coleman and colleagues introduced another mutant mouse (mutation diabetes; *db/db* or *Lep^{db}*) with marked hyperphagia, obesity, and severe diabetic symptoms such as hyperglycemia, polyuria, and glycosuria [3]. As a result of a series of symbolic “parabiosis experiments” by Coleman and colleagues, it was realized that

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Lep^{r^{db}} mice overproduced a certain circulating factor but were unable to respond to it, whereas the *Lep^{ob}* mice responded, but could not synthesize this factor [4, 5]. Using a positional cloning technique, Friedman's group cloned this circulating factor and named it "leptin" [6]. The initial belief that leptin could cure human obesity was thwarted, however, by the fact that most obese individuals are hyperleptinemic with intact leptin receptors and did not lose weight after leptin treatment, so called leptin resistance. It was shown however, similar to a classical endocrine replacement therapy, that the administration of recombinant leptin to both leptin deficient humans and rodents dramatically reduced excessive body weight, hyperphagia, and hyperglycemia [7, 8].

During the past few decades, the leptin research field has dramatically expanded into a wide range of studies that have explored not only the leptin signaling pathways but also the precise site and the function of leptin action and the mechanisms of leptin resistance. Initial findings indicated the CNS as the major action site of leptin action as the brain-specific deletion or reconstitution of leptin receptors demonstrated the sufficiency and requirement of CNS leptin action for the regulation of energy homeostasis [9, 10]. Recent studies have also shown the effects of leptin in diverse sites both in the CNS and in the periphery [11–13]. However, the functional roles of endogenous leptin in peripheral organs have largely remained obscure. In our current review, we seek to provide a comprehensive overview on the various mechanisms through which leptin activates and interacts with intracellular signaling pathways in the hypothalamic neurons.

Leptin and its receptors (Fig. 1)

Leptin is a cytokine-like hormone predominantly produced in the white adipose tissue and secreted into the systemic circulation [6]. Leptin mRNA expression is also found in other tissues including brown adipose tissue, placenta, ovary, skeletal muscle, stomach, pituitary gland, and the CNS [14–21], suggesting the local production of leptin in these organs. Leptin production in various tissues may be suggestive of pleiotropic effects of leptin on various biological functions. In addition to its critical role in appetite regulation and energy balance, leptin modulates immune function, gonadal function, and stress responses [22–24].

Leptin belongs to the long-chain helical cytokine family [25] and exerts its regulatory effects on energy homeostasis through its receptors which have a wide distribution in the body, particularly in the CNS, in both humans and rodents [26]. The leptin receptor (LepR) is a single membrane spanning receptor belonging to the class 1 cytokine

receptor family. There are multiple leptin receptor isoforms that are expressed from the primary leptin receptor gene by alternative splicing [27, 28]. The leptin receptor gene was cloned from the murine choroid plexus using tagged proteins to identify cells with leptin binding activity [29]. The identification of the leptin receptor in the brain was a major breakthrough in our understanding of leptin biology as it provided further insights into the mechanism regulating leptin activity and paved the way for further studies on the obese and hyperphagic phenotypes observed in *Lep^{ob}* and *Lep^{r^{db}}* mice [25, 27, 30].

The leptin receptor gene contains 17 common exons and several alternatively spliced 3'-exons [31]. To date, six isoforms of the leptin receptor have been identified in rodents (LepRa–f), all possessing the extracellular domains necessary for ligand binding [6, 32] (Fig. 1). LepRs can be categorized into three groups: secreted, short, and long isoforms. The secreted isoforms, such as LepRe, are either products of alternatively spliced messenger RNA species (in the mouse) or proteolytic cleavage products of the membrane bound form (in human) [31, 32]. LepRe is the smallest isoform and contains neither the cytoplasmic nor the transmembrane domains but only the extracellular domains that can bind to circulating leptin [33].

The short form LepRs include LepRa, c, d and f in rodents, whereas LepRb is classified as a long form (Fig. 1). Leptin binds to the short and long form receptors with the same affinity. However, only the long form has the ability to transmit leptin signaling and initiate the necessary intracellular responses [34, 35]. The short and long forms of the leptin receptor are distinguished by their intracellular domain size and the presence of JAK/STAT binding sites. The long form (LepRb) has a large intracellular domain consisting of 304 residues and has several putative JAK and STAT binding sites, whilst the short form (LepRa) comprises a 34 residue-intracellular domain and only one putative JAK binding domain [36–40]. Leptin regulation of JAK/STAT signaling will be further described in a later section. Both the short and long forms contain exons 1–17 of the leptin receptor gene and share extracellular and transmembrane domains as well as the first 29 amino acids of the cytoplasmic domain [41]. In rodents, LepRa, c, and d have different terminal exons, encoding only 3, 5, and 11 amino acids, respectively, whereas the terminal exon of LepRb has 273 amino acids [31]. The shorter isoforms, LepRa, c, d, and f, have truncated cytoplasmic domains with reduced signaling transduction capability. The leptin receptor short isoforms have been identified in multiple species. LepRa–c are widely spread receptors. In contrast, LepRd is found only in mice and LepRf is expressed only in rats. However, the roles of the short form leptin receptors remain to be clearly elucidated.

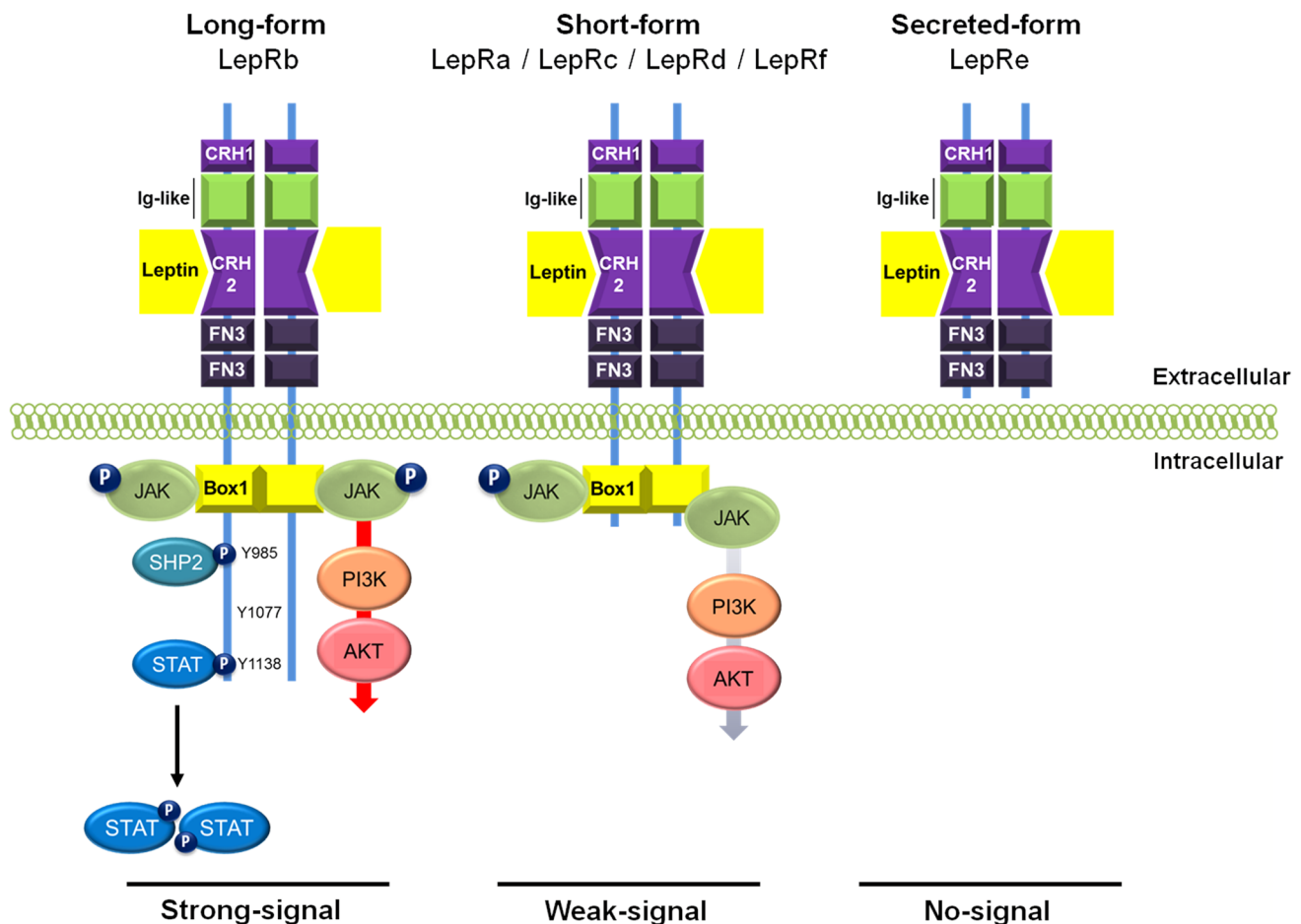


Fig. 1 Schematic representation of mouse leptin receptor isoforms. The mouse leptin receptor has six isoforms, LepRa–f with a common extracellular domain but diverse intracellular domains. JAK

interaction and activation is mediated through the box 1 motif. The long form, LepRb, is the only isoform that contains motifs enabling the leptin-induced activation of the JAK–STAT pathway

Despite the normal expression of the short form leptin receptors in *Lep^{r^{db}}* mice, these mice manifest an obese phenotype, indicating that the short form receptor cannot mediate leptin-induced changes in energy homeostasis [42]. Recent reports have described the phenotype of the LepRa knockout mouse [43, 44]. These mice have slightly lower fasting blood glucose level and improved glucose tolerance with normal body weight and food intake under chow diet-fed conditions. However, when given a high fat diet, they develop mild obesity along with modest leptin resistance and decreased leptin transport into the cerebrospinal fluid. Thus LepRa seems to mediate some of the effects of leptin, but its role in metabolic regulation appears to be limited when compared to LepRb. Further studies are needed to address the importance of the short form leptin receptors in energy homeostasis. It will also be necessary to establish whether there is any interaction between the short form and the long form leptin receptors in mediating the anorectic effects of leptin in the brain.

Signaling pathways that transduce the hypothalamic actions of leptin (Fig. 2)

JAK–STAT signaling

JAK–STAT signaling is a representative signaling pathway through which leptin regulates food intake and energy homeostasis in the hypothalamus. Leptin binds to its receptors and initiates downstream signaling through the sequential phosphorylation of the tyrosine kinase Janus kinase (JAK) and the transcription factor signal transducer and activators of transcription (STAT). The phosphorylation of STAT induces its dimerization and translocation to the nucleus, where it binds DNA and modulates the transcription of genes involved in food intake and energy homeostasis [45].

STAT molecules are cytoplasmic proteins activated by numerous factors including cytokines, growth factors, and hormones including leptin. The mammalian STAT family has seven members, STAT1–4, STAT5a, STAT5b and

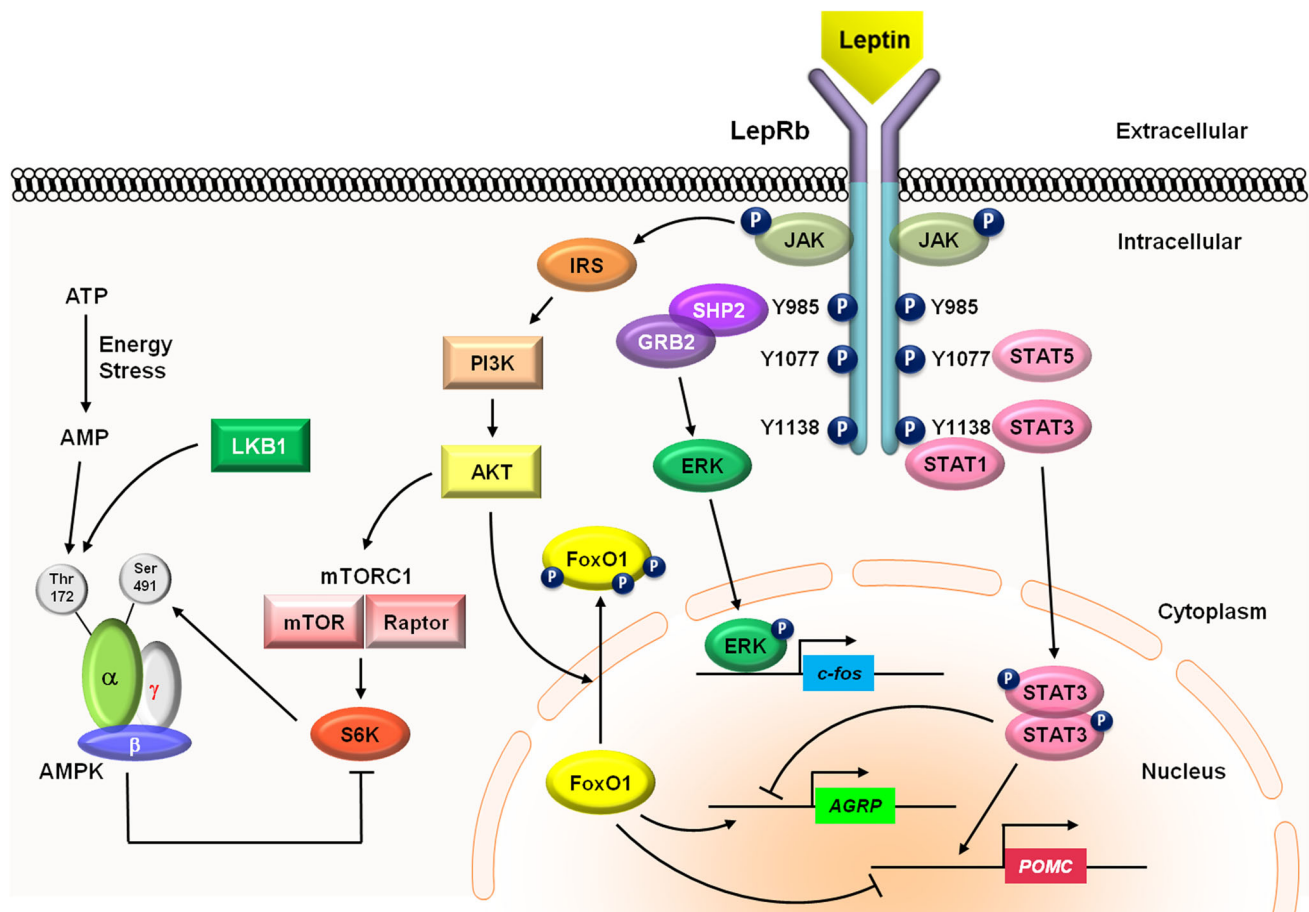


Fig. 2 Signaling pathways of leptin and its downstream effectors. Leptin binds to its receptor and activates the JAK–STAT3, PI3K–FoxO1, and ERK pathways. For JAK–STAT activation, activated JAK2 tyrosine kinase induces the phosphorylation of Tyr985 and Tyr1138 of LepRb, which leads to the activation of STAT3/STAT5. Phosphorylated Tyr985 strongly recruits SHP-2 and GRB2 resulting in the activation of ERK signaling. Leptin also activates PI3K by recruiting IRS proteins leading to the inactivation of FoxO1 by sequestering them in the cytoplasm. On the other hand, leptin has

been reported to inhibit AMPK activity. AMPK is activated by upstream signaling, including LKB1 and CaMKK β . In addition, leptin treatment activates mTOR/S6K signaling in the hypothalamus, which phosphorylates Ser491 of AMPK α -subunit and inhibits AMPK activity. *AgRP* agouti-related protein, *FoxO1* forkhead box protein O1, *IRS* insulin receptor substrate, *JAK* Janus kinase, *PI3K* phosphatidylinositol 3-OH kinase, *POMC* proopiomelanocortin, *PTP1B* protein-tyrosine phosphatase 1B, *SOCS3* suppressor of cytokine signaling 3, *STAT* signal transducer and activator of transcription

STAT6. Leptin induces the phosphorylation of STAT1, STAT3, STAT5 and STAT6, among which STAT3 and possibly STAT5 mediate leptin-induced anorectic effects [34, 46–50].

STAT3 is widely expressed in the CNS. Mouse models have been employed to explore the role of STAT3 in leptin regulation of energy homeostasis [50]. Neuron-specific STAT3 deletion resulted in hyperphagia, severe obesity, diabetes, and hyperleptinemia in mice [51], traits which were also observed in leptin- and LepRb-deficient mice and classified as hallmarks of leptin resistance [52, 53]. Phosphorylated STAT3 facilitates the leptin-mediated transcriptional regulation of key appetite-regulating neuropeptides such as POMC, AgRP, and NPY. POMC is a precursor peptide which is cleaved by prohormone convertases, giving rise to anorexigenic α -melanocyte

stimulating hormone (α -MSH). AGRP and NPY are potent orexigenic peptides synthesized in discrete neurons of the hypothalamic ARC. Leptin increases the transcriptional activity of POMC whereas a dominant-negative form of STAT3 inhibits this activity, indicating that POMC is a major target gene of LepRb–STAT3 signaling [54]. Increased food intake in STAT3 mutant mice can be attributed to the dysregulated transcription of POMC, AgRP and NPY genes [55, 56].

Tyrosine 1138 of LepRb mediates STAT3 activation during leptin action [57] and its substitution for serine (*Lepr*^{S1138}) specifically disrupts LepRb–STAT3 signaling. Similar to *Lepr*^{db} mice, mice harboring the *Lepr*^{S1138} allele (*s/s* mice) are hyperphagic and obese from an early age. In these animals, obesity progresses throughout adulthood and is accompanied by elevated plasma leptin levels, implying

leptin resistance [50]. *Lepr^{db}* mice are also infertile, short, and diabetic whereas *s/s* mice display normal fertility and growth and less severe hyperglycemia. Furthermore, hypothalamic POMC expression is similarly suppressed in both *Lepr^{db}* and *s/s* mice, whereas NPY expression is elevated in *Lepr^{db}* mice but not in *s/s* mice. Hence, the LepRb–STAT3 signaling pathway is critical for the effects of leptin on melanocortin production and body energy homeostasis, but other signaling pathways may transduce actions of leptin on the hypothalamic NPY, on neuroendocrine functions such as fertility and growth, and on glucose metabolism [50].

Leptin administration increases the transcription of suppressor of cytokine signaling 3 (SOCS3) in the hypothalamus, via tyrosine 1138 of LepRb, whilst SOCS3 is known to inhibit leptin activities. Thus a negative feedback mechanism exists in leptin-induced STAT3 signaling, through the induction of SOCS3 [58]. Leptin phosphorylates and activates STAT3, which then binds to two sites within the SOCS3 promoter to increase the transcription of SOCS3 [59]. Increased SOCS3 expression creates a feedback loop curtailing further activation of leptin–STAT3 signaling [60]. As such, the JAK–STAT3 pathway may be vital for the maintenance of energy homeostasis through the fine tuning of a modulatory loop in the molecular network of leptin action. Enhanced hypothalamic SOCS3 expression under obese conditions has been suggested as a major mechanism limiting leptin signaling and actions, which will be further discussed in the following section.

LRP1 and LRP2 (low-density lipoprotein receptor-related protein-1, 2) are multi-ligand endocytic receptors that belong to the LRP family of proteins [61]. Mice lacking neuronal LRP1 develop leptin resistance and obesity [62]. Moreover, a molecular interaction between LRP1 and leptin–LepRb complex has been demonstrated in the hypothalamus [62], indicating an involvement of LRP1 in hypothalamic leptin signaling. On the other hand, LRP2, also known as megalin/gp330, has been implicated in leptin reuptake in the renal tubules and leptin transport in the choroid plexus [63–65]. A blockade of hypothalamic LRP and endocytosis impairs leptin-induced anorexia and hypothalamic STAT3 phosphorylation [66, 67]. Thus, the LRP-mediated endocytosis of leptin–leptin receptor complex may be required for leptin-activated STAT3 signaling.

PI3K–Akt–FoxO1 signaling

The intracerebroventricular (ICV) administration of leptin activates the insulin receptor substrate (IRS)–phosphatidylinositol 3-OH kinase (PI3K) signaling in the mediobasal hypothalamus (MBH) [68]. Involvement of the IRS–PI3K pathway in leptin activity was first delineated

from the IRS2-null mouse phenotype, which involved an elevated leptin level, increased food intake and decreased energy expenditure [69]. Subsequently, it was shown that inhibiting the hypothalamic PI3K pathway suppressed the anorexigenic effects of leptin [68], suggesting a crucial role of PI3K in mediating leptin actions. The Sar homology family member SH2B1 mediates leptin-induced PI3K activation. SH2B1 enhances JAK2 activity and recruits IRS proteins to JAK2, which leads to JAK2-mediated IRS phosphorylation and subsequent PI3K activation [70, 71].

In hypothalamic neurons, PI3K integrates insulin and leptin signaling [72]. Leptin directly increases PI3K activity in POMC neurons, but indirectly inhibits it in AGRP neurons. By contrast, insulin activates PI3K signaling in both neuronal cell types [73]. PI3K activation mediates the acute effects of leptin on the neuroelectrical activity of POMC neurons as PI3K inhibitors blocks leptin-induced neuroelectrical changes in POMC neurons [74]. Disruption of PI3K signaling in POMC cells abolishes membrane depolarization and decreases the firing rate in response to leptin [74]. Nevertheless, mice with impaired PI3K signaling in POMC neurons have normal body weights. Thus PI3K signaling in POMC neurons is not critical for the leptin-mediated regulation of energy balance.

PI3K catalyzes the phosphorylation of phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-trisphosphate (PIP₃) whereas tumor suppressor phosphatase and tensin homolog (Pten) promotes the opposite reaction [75]. Activation of PI3K in LepR expressing neurons by ablation of Pten leads to a reduced body fat mass via increased sympathetic outflow to perigonadal white adipose tissue (WAT) resulting in brown adipose tissue-like transdifferentiation of WAT [76]. On the other hand, mice lacking Pten in POMC neurons develop obesity and leptin resistance through the activation of ATP-dependent K⁺ channels [77]. Thus chronic elevation of PIP₃ in POMC neurons may interfere with hypothalamic leptin activity.

The transcription factor forkhead box protein O1 (FoxO1) is a well-known phosphorylation target of PI3K downstream kinase Akt. FoxO1 mediates the anorectic effects of leptin and insulin by regulating the transcription of POMC and AgRP [78–81]. FoxO1 expression is found in the ARC, and ventromedial and dorsomedial hypothalamus, regions which also express leptin receptors. Under nutrition deprivation conditions, FoxO1 translocates from the cytoplasm to the nucleus where it increases the expression of the orexigenic NPY/AgRP genes and suppresses expression of anorexigenic POMC, thereby increasing food intake [78, 82]. Leptin hampers the FoxO1-mediated transcriptional regulation of POMC, NPY and AgRP [78, 82, 83]. Conversely, activated FoxO1 inhibits the ability of leptin to induce anorexia and to stimulate

POMC transcription [83]. In line with this, mice with depleted FoxO1 in the POMC neurons are more sensitive to the anorectic effects of leptin [84]. These data suggest that reduced FoxO1 activity via leptin is important for leptin-mediated feeding regulation.

There seems to be a crosstalk between STAT3 and FoxO1 signaling. The overexpression of FoxO1 interrupts STAT3-stimulated POMC promoter activity [85]. FoxO1 does not inhibit leptin-mediated STAT3 phosphorylation nor does it block the translocation of the activated STAT3 to the nucleus. Instead, FoxO1 inhibits the interaction between STAT3 and the SP1 transcription factor, which critically mediates leptin-induced POMC transcriptional activity [85].

SHP2–ERK signaling

The mitogen-activated protein kinases (MAPKs) function to regulate numerous intracellular programs, including cell proliferation and differentiation, by relaying extracellular signals to the cell. The involvement of hypothalamic extracellular signal-regulated kinase (ERK) signaling in energy homeostasis was suggested by ERK activation in the ARC and paraventricular nucleus (PVN) under nutrition deprivation conditions [86] which was reversed after re-feeding [87]. Several studies have demonstrated the role of MAPKs, particularly ERK1/2, in the leptin regulation of energy homeostasis [57, 88, 89]. Leptin activates ERK1/2 but not p38 MARK in the hypothalamic ARC. Furthermore, a pharmacological blockade of hypothalamic ERK1/2 reverses the anorectic and weight-reducing effects of leptin and also inhibits leptin-induced thermogenesis by controlling sympathetic activity on BAT. These findings indicate that the hypothalamic ERK plays an important role in the control of energy balance by leptin [90].

LepRb mediates ERK activation as evidenced by the leptin induced ERK activation in the lean Zucker rat but not in the obese Zucker rat with a defective LepRb [90]. Among the Tyr 985, Tyr 1077 and Tyr 1138 residues of LepRb, Tyr 985 is implicated in leptin activated ERK signaling. The deletion of Tyr 985 inhibits leptin-induced ERK activation by about 70 %, indicating the involvement of another mechanism in ERK activation such as through the leptin receptor short form [57, 91]. ERK1 and 2 are typically activated through the receptor tyrosine kinase upon ligand binding. The activation and autophosphorylation of the tyrosine residue in the intracellular domain of LepR facilitates its interaction with adapter proteins containing the Src homology 2 (SH2) domain [92]. SHP2, a SH2-containing tyrosine-specific protein phosphatase, appears to be a key modulator in leptin–ERK signaling as the phosphatase activity of SHP2 is necessary for leptin-mediated ERK activation [93].

Leptin plays a significant role in the early development of hypothalamic feeding circuits [94]. ARH projections to the PVN, dorsomedial nucleus (DMN), and lateral hypothalamus are markedly reduced in leptin deficient *Lep^{ob}* mice and restored by leptin treatment during the neonatal period. Both ERK and STAT3 signaling were found to be important for leptin-stimulated formation of hypothalamic neuronal circuits as disrupted ERK and STAT3 signaling impaired ARH projections [95]. Recently, it was reported that ERK signaling in hypothalamic tanycytes is crucial for leptin transport to the MBH [96]. Activation of ERK signaling with epidermal growth factor (EGF) restores decreased leptin transport and leptin sensitivity in the hypothalamus of diet-induced obese animals.

AMPK signaling

Adenosine monophosphate-activated protein kinase (AMPK) is an intracellular energy sensor activated under conditions of metabolic stress causing ATP depletion and an intracellular increase in AMP and ADP levels [97]. Once activated, AMPK restores energy balance by initiating ATP generating catabolic processes such as fatty acid oxidation and suppressing ATP consuming anabolic processes. AMPK is a heterotrimeric serine/threonine kinase consisting of a catalytic α subunit and regulatory β and γ subunits [98, 99]. AMPK activation occurs through the phosphorylation of threonine 172 on the catalytic α subunit by the upstream kinase Peutz–Jegher syndrome tumor-suppressor gene product LKB1 and Ca^{2+} /calmodulin-dependent protein kinase kinase- β (CaMKK β) [100, 101].

AMPK plays a central role in energy homeostasis by integrating hormonal and nutritional signals in both the periphery and the brain. In the hypothalamus, AMPK is activated by fasting but suppressed by refeeding. Leptin inhibits AMPK activity in distinct hypothalamic regions such as the ARC and PVN, hence reducing food intake and body weight [102]. A reduction in AMPK phosphorylation by leptin administration is followed by decreased phosphorylation of the AMP downstream target acetyl-CoA carboxylase (ACC) and elevated malonyl-CoA levels in the hypothalamic neurons [103]. Increased malonyl-CoA reduces mitochondrial fatty acid oxidation by suppressing the carnitine palmitoyl transferase (CPT)-1 activity, thereby increasing the cellular long chain fatty acyl-CoA (LCFA-CoA) level. Increased LCFA-CoA content in the hypothalamus is known to induce anorexia and reduce hepatic glucose production [104]. On the other hand, systemic administration of leptin stimulates skeletal muscle AMPK activity and enhances fatty acid oxidation through both direct and CNS-mediated indirect mechanisms [105]. Hypothalamic AMPK signaling also transduces the effects

of leptin on the sympathetic nerve outflows to the kidney, brown and white adipose tissues [106].

Extensive studies have also revealed phosphorylation sites in the α and β subunits that alter the activity of AMPK [107, 108]. Of the two catalytic subunits, $\alpha 1$ and $\alpha 2$, leptin appears to modulate the activity of AMPK by acting on the $\alpha 2$ -AMPK subunit [102]. Hypothalamic $\alpha 2$ -AMPK activity is important for homeostatic regulation of energy balance. ICV administration of leptin in wild type mice increased the phosphorylation of $\alpha 2$ -AMPK at Ser⁴⁹¹ in the hypothalamus which decreased $\alpha 2$ -AMPK activity. Similarly, ICV injection of leptin had no significant effects on the body weight or food intake of rats with an siRNA silenced $\alpha 2$ AMPK subunit [106]. Mice lacking $\alpha 2$ -AMPK in POMC neurons displayed obesity due to reduced energy expenditure and increased food intake whilst an $\alpha 2$ -AMPK deletion in AgRP neurons resulted in an age-dependent lean phenotype [109]. Phosphorylation of Ser⁴⁹¹ on $\alpha 2$ -AMPK by p70 S6 kinase (S6K) has been suggested as the mechanism underlying the leptin-mediated inhibition of AMPK.

mTOR–S6K signaling

The mammalian target of rapamycin (mTOR) is an evolutionally conserved serine–threonine kinase, which senses nutrient (especially amino acids) availability, stimulates protein synthesis, cell growth and proliferation, and inhibits autophagy [110]. Like AMPK, mTOR serves as a cellular fuel sensor [111]. mTOR and its downstream target, S6K1 are expressed in hypothalamic neurons including NPY/AgRP neurons and POMC neurons [112]. Leptin administration acutely increases hypothalamic mTOR activity, determined by increased phospho-S6K1 and phospho-S6 ribosomal protein. Moreover, the coadministration of the mTOR inhibitor rapamycin significantly blocks the anorectic effects of leptin. Mice lacking S6K1 failed to reduce their food intake in response to leptin [113]. Furthermore, S6K activity in the MBH affected hypothalamic leptin sensitivity. Leptin-induced reduction in body weight and food intake is more pronounced in rats expressing constitutively active S6K1 in the MBH whereas it was found to be mitigated in rats expressing dominant negative (DN)-S6K1 [114]. All of these data support an indispensable contribution of mTOR–S6K signaling in the hypothalamic actions of leptin. In addition to feeding regulation, ARC mTOR activation by leptin is important for the sympathetic and cardiovascular effects of leptin [115]. Both rapamycin administration and hypothalamic DN-S6K expression prevent the stimulatory effects of leptin on renal sympathetic nerve outflow and arterial blood pressure. In high-fat-diet-fed animals, leptin is unable to modulate hypothalamic mTOR activity [112],

implying the development of leptin resistance in this signaling pathway.

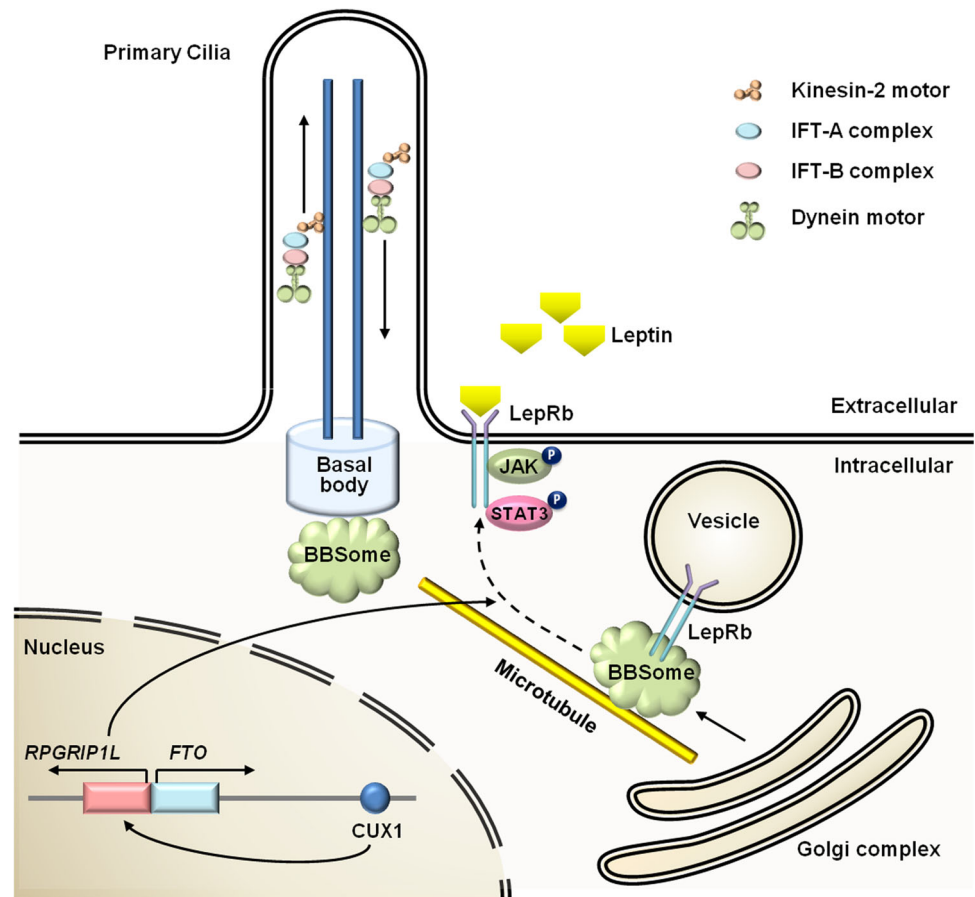
In the hypothalamic neurons, leptin activates mTOR–S6K1 through the activation of PI3K–Akt signaling. As mentioned in the previous section, activated S6K1 in turn phosphorylates $\alpha 2$ -AMPK at serine 491 which leads to reduced $\alpha 2$ -AMPK activity in the MBH [116]. Thus mTOR–S6K signaling serves as an important pathway upstream of AMPK in the hypothalamic leptin signaling cascades. In contrast to its role in leptin signaling, chronic mTOR activation induced by depleting TSC1 and by aging in POMC neurons causes hyperphagic obesity. Interestingly, rapamycin and hypothalamic S6K1 inactivation successfully treated activated mTOR-induced obesity [117–119]. Thus the metabolic consequences of altered mTOR signaling may differ depending on neuronal cell type and metabolic context.

Cilia-related signaling (Fig. 3)

A cilium is a tiny hair-like organelle projecting from the cell surface. Cilia consist of a 9+2 or 9+0 microtubule-based axonemal complexes, which are assembled on a basal body and covered with a specialized plasma membrane. Most mammalian cells have single immotile (primary) cilia, which have been implicated in various cellular signaling events [120]. Several lines of evidence suggest that leptin signaling is closely tied to the cilia–basal body complex. Obesity is one of the cardinal manifestations of human ciliopathy Bardet–Biedl syndrome (BBS) [121]. Likewise, murine BBS models lacking BBS2, BBS4, and BBS6 proteins display obesity [122]. In these mice, leptin-induced hypothalamic STAT3 phosphorylation, anorexia, and weight loss are severely impaired, even upon calorie restriction to avoid leptin resistance secondary to obesity. These data have suggested that BBS proteins may be involved in hypothalamic leptin signaling and actions. Seven BBS proteins (BBS1, BBS2, BBS4, BBS5, BBS7, BBS8 and BBS9) form a stable complex known as the BBSome [123], which mediates protein/vesicle trafficking to cilia. Interestingly, LepRb specifically interacts with BBS1 [122]. Furthermore, the depletion of BBS1 or BBS2 in retinal pigment epithelial cells disrupts LepRb trafficking to the post-Golgi network (PGN), resulting in aberrant accumulation of LepRb-containing vesicles in the perinuclear area [122]. Therefore, the BBSome might interact with LepRb to mediate LepRb trafficking to the PGN.

Another ciliary gene, retinitis pigmentosa GTPase regulator-interacting protein-1 (RPGRIPL) has been shown to coordinate leptin signaling in hypothalamic neurons [124, 125]. RPGRIPL-haploinsufficient mice exhibit increased fat mass and food intake and reduced energy

Fig. 3 Association of cilia signaling and leptin signaling. Primary cilia are associated with leptin signaling in hypothalamic neurons. BBS proteins form a protein complex called BBSome, which is involved in the trafficking of vesicles laden with ciliary proteins from the Golgi to cilia–basal bodies. The BBSome, especially BBS1, may mediate the trafficking of LepRb to the cellular surface or periciliary area as the deletion of BBS1 causes the aberrant distribution of LepRb in the perinuclear area. Another ciliary gene, RPGRIP1L, interacts with LepRb and mediates LepRb trafficking to the periciliary area. RPGRIP1L expression is regulated by CUX1, which binds to the first intron of FTO gene and stimulates the transcription of RPGRIP1L and FTO. BBS Bardet–Biedl syndrome, CUX1 cut-like homeobox 1, FTO fat mass and obesity associated, RPGRIP1L retinitis pigmentosa GTPase regulator-interacting protein-1 like



expenditure [125]. These mice show reduced anorectic responses and hypothalamic STAT3 activation by exogenous leptin, even before the development of obesity. Like BBS proteins, RPGRIP1L interacts with LepRb and mediates its trafficking to the periciliary area. In LepRb-expressing normal fibroblasts, LepRb localizes to the vicinity of cilium upon leptin treatment [125]. In line with this, LepRb colocalizes with the PGN markers TGN46 and ARF4 near the base of cilium in the hypothalamic arcuate nuclei of mice following leptin treatment. LepRb fails however to locate around the base of the cilium in fibroblasts with an RPGRIP1L mutation and in RPGRIP1L-haploinsufficient mice. Thus, RPGRIP1L-mediated LepRb trafficking to the TGN and periciliary area may be critical in hypothalamic leptin signaling. Interestingly, the expression of RPGRIP1L was found to be regulated by the cut-like homeobox 1 (CUX1), which binds to the first intron of the fat mass and obesity associated (FTO) gene and stimulates the transcription of RPGRIP1L and FTO. Common single nucleotide polymorphisms (SNPs) in the FTO gene are significantly associated with the fat mass in humans [126, 127] and have been shown to disrupt CUX1-stimulated RPGRIP1L and FTO transcription. Hence, common variants in the FTO gene may be

attributable to adiposity through the impairment of leptin receptor trafficking to the cilia in hypothalamic neurons.

Loss of cilia in neurons, especially in POMC neurons, by depleting the intraflagellar transport protein 88 homolog (IFT88) gene leads to hyperphagic obesity and hyperleptinemia [128]. Likewise, the intrahypothalamic injection of small inhibitory RNAs targeting kinesin-2 subunit KIF3A and IFT88 significantly reduces the effects of exogenous leptin on food intake and hypothalamic STAT3 phosphorylation [129]. Mice lacking the type 3 adenylyl cyclase (AC3), used in the immunostaining of neuronal cilia, were found to be obese and hyperleptinemic [130]. All of these data suggest that neuronal cilia and ciliary proteins may be required for leptin signal transduction and/or the maintenance of an energy balance. In contrast, a recent study has reported that IFT88- and BBS4-knockout mice normally respond to leptin under preobese conditions [131], suggesting a dispensable role of the cilia in hypothalamic leptin signaling. Further studies are needed to clarify how cilia influence leptin signaling processes.

On the other hand, leptin regulates the cilia lengths in hypothalamic neurons. The average lengths of hypothalamic cilia are shorter in *Lep^{ob}* and *Lep^{db}* mice compared to normal mice [129]. In line with this, a 7 day-leptin

treatment of *Lep^{ob}* mice rescued the short cilia phenotype. The control of hypothalamic neuron cilia lengths by leptin was found to be mediated through PTEN–GSK3 β signaling which involves ciliary gene transcription and actin cytoskeleton rearrangement [132]. Thus it is plausible that leptin modulates its own signaling by promoting cilia growth in hypothalamic neurons.

Signaling pathways that inhibit the actions of leptin (Fig. 4)

The discovery of leptin generated considerable attention and expectations for its putative use as a therapeutic agent against human obesity. However, obese individuals were found to be refractory to leptin therapy in clinical trials [133, 134]. In fact, obese individuals generally exhibit elevated circulating leptin concentrations in proportion to their increased body fat mass [135]. Thus, an

understanding of why an increased leptin level under obese conditions does not yield the expected effects is essential for any future strategies to combat human obesity in this way.

The number of publications per year citing ‘leptin resistance’ has been growing steadily since this hormone was discovered [136]. In clinical settings, the term “leptin resistance” can be defined literally as the inability of exogenous leptin to promote desired outcomes including reduced appetite and body weight. In contrast, in many other situations, the meaning of this term is considered to be hyperleptinemia accompanied by obesity, which reflects expanded adipose tissue [136].

Leptin resistance emanates from reduced leptin transport into the brain or defects in *LepRb* signaling cascades in the hypothalamic neurons. Leptin-induced activation of hypothalamic neurons, including ARC, ventromedial hypothalamus (VMH), and lateral hypothalamic areas, can be traced by immunohistochemical analysis of

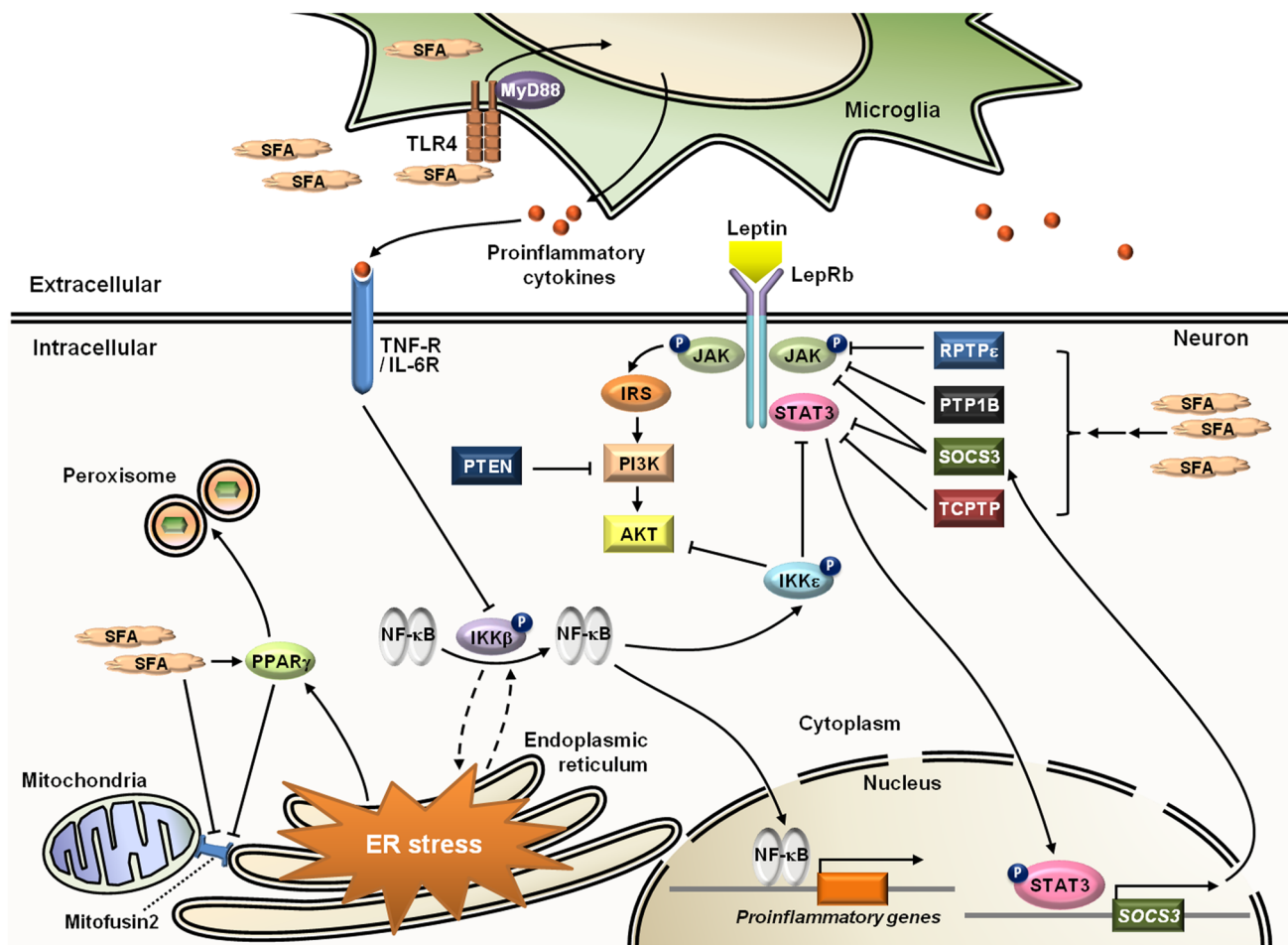


Fig. 4 Signaling molecules which negatively regulate leptin signaling. Several signaling pathways are activated in the hypothalamus of DIO mice and attenuate signaling cascades downstream from the leptin receptors via interactions with JAK2, STAT3, IRS2, and PI3K.

IKKβ I κ B kinase- β , *NF κ B* nuclear factor- κ B, *PKC- θ* protein kinase- θ , *PPAR γ* peroxisome proliferator activated transcript- γ , *PTP1B* protein tyrosine phosphatase 1B, *SOCS3* suppressor of cytokine signaling 3

phosphorylated STAT3 [137]. Upon administration of a pharmacological dose of leptin, diet-induced obesity (DIO) rodents show a limited amplitude of leptin signaling in the hypothalamus, as evidenced by reduced STAT3 phosphorylation compared with lean animals [138]. Putative negative regulators of leptin receptor signaling are described below.

SOCS3

In the hypothalamic neurons, activated STAT3 in response to leptin signaling induces SOCS3 expression, which in turn yields a negative feedback loop by binding to the LepRb–JAK2 complex and thereby attenuating leptin signaling [139]. Since hypothalamic SOCS3 expression was found to be elevated in leptin-resistant obese yellow (*A^{y/a}*) mice, an increased SOCS3 level was the first proposed mechanism of leptin resistance [139]. A role of SOCS as a negative regulator of leptin signaling was confirmed by the fact that obesity and leptin resistance upon high-fat diet (HFD) feeding are less severe in SOCS3 heterozygous knockout mice as well as in mice with neuronal SOCS3 deletion [140–142]. Consistently, increased SOCS3 expression in POMC neurons led to impaired STAT3 signaling, leptin resistance and obesity. However, SOCS3 overexpression in LepR-expressing neurons did not cause obesity, suggesting that the impacts of SOCS3 signaling on energy balance may be neuron type-specific [143].

ARC SOCS3 expression increases from 1 week after HFD feeding, ahead of the establishment of DIO [53, 144, 145]. During the development of DIO, SOCS3 activation in AgRP neurons precedes that in POMC- and other hypothalamic neurons [146]. From these findings, it appears that the HFD-induced increase of SOCS3 may have a distinct temporal and spatial pattern and AgRP neurons may be the predominant early responders to subtle changes in leptin signaling upon short-term HFD feeding [146].

A recent paper has shown that the central and peripheral administration of high-dose leptin receptor antagonist induces comparable changes in food intake, body weight, and hypothalamic POMC and SOCS3 mRNA levels in lean and obese mice [53, 144, 145]. Thus endogenous leptin signaling may not be much reduced in obese mice on a HFD, which challenges the previous concept of leptin resistance under obese conditions. Instead, elevated hypothalamic SOCS3 expression in DIO mice may limit additional activation of LepRb signaling by exogenous leptin [147].

PTP1B and other tyrosine phosphatases

Protein tyrosine phosphatase-1B (PTP1B) is a cytoplasmic enzyme predominantly localized in the endoplasmic reticulum (ER) [148]. PTP1B is expressed in hypothalamic

areas such as the ARC, VMH, and dorsomedial hypothalamus (DMH) [149]. Like SOCS3, PTP1B inhibits LepRb signaling by targeting JAK2. PTP1B binds to and dephosphorylates JAK2, thereby attenuating STAT3 tyrosine phosphorylation in the hypothalamus [149–151]. PTP1B also negatively regulates hypothalamic leptin signaling via α 2-AMPK [152]. Consistent with these mechanisms, mice with a mutation or depletion of PTP1B in hypothalamic neurons are leaner, more sensitive to leptin and resistant to DIO and display improved glucose homeostasis [138, 152–156], supporting an antagonistic effect of PTP1B in hypothalamic leptin signaling. The impact of PTP1B on energy balance appears to rely on hypothalamic LepR signaling as hypothalamic PTP1B depletion leads to reduced adiposity, an effect that is abolished by the concomitant hypothalamic deletion of LepR [157].

Previous studies have revealed increased PTP1B expression in the MBH of rodent DIO models [156, 158, 159] although one study has reported an unaltered hypothalamic PTP1B level during HFD feeding [144]. Aging is also related to elevated MBH PTP1B expression [160]. Hyperleptinemia may contribute to an obesity-associated increase in hypothalamic PTP1B as chronic leptin treatment significantly increases the hypothalamic PTP1B mRNA level [155, 159].

Several molecules are involved in hypothalamic PTP1B signaling. Coexpression of PTP1B with SH2B1 blocks the inhibitory effect of PTP1B on leptin-induced STAT3 phosphorylation [71]. LIM domain Only 4 (LMO4), a transcription cofactor involved in STAT3 activation in VMH neurons [161, 162], downregulates PTP1B activity by augmenting the PTP1B inactive form via oxidation [163]. There have been trials to treat obesity and leptin resistance by inhibiting PTP1B. Trodusquemine, a spermine metabolite of cholesterol, and its polyaminosteroid derivative claramine, cause a loss of fat mass in DIO mice by inhibiting PTP1B [164, 165].

Other PTPs may also participate in leptin receptor signaling. Tyrosine phosphatase ϵ (RPTP ϵ) dephosphorylates JAK2 and thus downregulates leptin receptor signaling in the hypothalamus. Leptin induces the phosphorylation and activation of RPTP ϵ at its C-terminal Y695 residue, which constitutes a negative feedback pathway of leptin signaling. Increased RPTP ϵ activity is found in the hypothalamus of obese, leptin-resistant mice [166]. Moreover, mice lacking RPTP ϵ are leptin-sensitive and protected from obesity induced by HFD, aging, and ovariectomy [166]. Another PTP variant, T cell PTP (TCPTP), is elevated in the hypothalamus of DIO mice where it negatively modulates leptin-induced STAT3 signaling. Genetic ablation or pharmacologic suppression of neuronal TCPTP enhances leptin sensitivity [167]. Other PTPs such as SHP2 or PTEN

may also have regulatory roles in leptin receptor signaling. Further studies are needed to elucidate the activities of these PTPs in LepRb signaling [168].

IKK–NF κ B signaling

De Souza et al. reported for the first time that a long-term high fat diet increases the hypothalamic expression of proinflammatory cytokines such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor- α (TNF α) and activates the inflammatory signaling pathways c-Jun N-terminal kinase (JNK) and nuclear factor- κ B (NF- κ B) in the rat hypothalamus. These authors suggested this phenomenon as a mechanism of hypothalamic insulin resistance [169]. Thereafter, the cumulative evidence has indicated that activated proinflammatory signaling in the hypothalamus is an important mechanism underlying HFD-induced leptin resistance. Notably, only 1 day on a HFD induced hypothalamic inflammation in rodents [170]. The key mediators of inflammation/innate immunity, I κ B kinase- β (IKK β) and NF κ B, are highly expressed in MBH neurons [171]. Activation of hypothalamic IKK β –NF κ B signaling is observed upon exposure to 1 day of HFD or following a single ICV administration of glucose or fatty acid [170–172]. Thus, HFD-induced hypothalamic inflammation may be an early event in the development of overnutrition-induced leptin resistance.

Artificial activation of hypothalamic IKK β –NF κ B signaling by viral mediated IKK β overexpression in the MBH increases food intake and promotes weight gain along with impaired leptin signaling. Conversely, the neuronal knockout of IKK β or the virus-mediated deletion of IKK β in MBH attenuates the development of DIO [171, 173, 174]. These results demonstrate a causative role of IKK β –NF κ B signaling in leptin resistance in DIO mice. Interestingly, the metabolic impacts resulting from activated IKK β –NF κ B signaling in POMC and AgRP neurons appear to be opposing. Inactivation of IKK β –NF κ B signaling in the AgRP neurons protects against obesity, suggesting the obesity promoting effect of NF κ B signaling in AgRP neurons [171]. However, a recent study has demonstrated that activation of IKK β signaling in AgRP neurons fails to cause obesity and leptin resistance but instead disrupts insulin signaling in AgRP neurons and impairs systemic glucose homeostasis [175]. In contrast, NF κ B signaling in POMC neurons is activated by leptin and mediates its anorexigenic effects [176]. NF κ B binding sites within the POMC promoter are hypermethylated in mice with HFD-induced obesity, which limits the ability of leptin to increase POMC expression [177].

In the hypothalamus, activation of IKK β –NF κ B signaling induced by overnutrition leads to increased expression of proinflammatory cytokines such as TNF α ,

IL-1 β , and IL-6 [169, 178, 179]. In support of a role of hypothalamic proinflammatory cytokines in overnutrition induced leptin resistance, the lack of TNF receptor-1 or central infusion of TNF α neutralizing antibody was found to mitigate leptin resistance under HFD conditions [180, 181]. Consistently, ICV coinjection of TNF α partially blocks the anorexigenic effect of leptin through the inhibition of PI3K–Akt–FoxO1 signaling [182]. On the other hand, central infusion of recombinant IL-6 increases hypothalamic STAT3 phosphorylation. Furthermore, IL-6 treatment of DIO mice suppresses hyperphagia and inhibits hypothalamic IKK β activation [183], suggesting beneficial metabolic effects of IL-6. Interestingly, injection of leptin increases the IL-1 β level in the hypothalamus of normal Sprague–Dawley rats [184]. A central injection of IL-1 receptor antagonist inhibits the satiety effect caused by central or peripheral leptin injection and abolishes the leptin-induced increase in body temperature. Mice lacking IL-1 receptor show no reduction in food intake in response to leptin, suggesting that IL-1 β acts as an important downstream mediator of hypothalamic leptin actions [184]. Thus, proinflammatory cytokines may have differential roles in hypothalamic leptin signaling [170, 185, 186].

Several molecules engaged in proinflammatory signaling pathway have been implicated in hypothalamic inflammation and leptin resistance. First, the Toll-like receptors (TLRs) are membrane-bound pattern recognition receptors, which play an important role in innate immune defense [187]. Saturated fatty acids serve as a naturally occurring ligand for TLR4, which mediates the fatty acid-induced activation of IKK β –NF κ B signaling [188]. Hypothalamic TLR4 expression and its downstream signaling activity are increased by both the acute ICV infusion of saturated fatty acids and chronic HFD feeding [178, 179]. Furthermore, inhibition and a loss-of-function mutation of TLR4 protects mice from DIO and reduces hypothalamic inflammation [179, 181]. These results demonstrate a critical role of TLR4 in diet-induced hypothalamic inflammation. As hypothalamic TLR4 expression is mostly found in activated microglia [179], interactions between microglia and hypothalamic neurons may be crucial for the progression of hypothalamic inflammation. Second, the myeloid-differentiation primary-response gene 88 (MyD88) is an essential signaling adaptor molecule that couples TLR4 to inflammatory signaling cascades [189]. Similarly to TLR4, hypothalamic MyD88 expression is stimulated by the ICV administration of saturated fatty acids [190]. Mice lacking MyD88 in the CNS are more sensitive to leptin upon acute and chronic exposure to fatty acids, indicating a role of MyD88 in the development of central leptin resistance [190]. Third, IKK ϵ is a downstream molecule of NF κ B signaling. Inhibition of IKK ϵ activity reduces leptin resistance by restoring JAK2–

STAT3 and IRS–PI3K–Akt signaling in the hypothalamus of HFD-fed mice [191].

Interestingly, endotoxin lipopolysaccharides (LPS) and leptin share a common signaling pathway in the hypothalamus. Similar to leptin, a single intraperitoneal injection of LPS increases hypothalamic STAT3 phosphorylation and POMC expression but decreases hypothalamic AMPK activity, thereby inducing a hypophagic response [192, 193]. Moreover, exposure to LPS acutely provokes an excitatory tone in most POMC neurons and an inhibitory tone in AgRP/NPY neurons. Blockade of the TLR4 receptor in microglia reduces the inhibitory effect of LPS on AgRP/NPY neurons, indicating an indirect and inhibitory regulation by LPS in these cells [194]. Repeated injection of LPS leads to a reduced response to LPS, implying the development of LPS resistance. Moreover, leptin-resistant DIO rats show a blunted hypophagic response to LPS whilst mice receiving a repeated LPS injection show reduced leptin sensitivity [195]. These findings indicate that the mechanisms underlying hypothalamic resistance to endotoxin largely overlap with those of leptin resistance.

ER stress–mitochondrial signaling

The rough endoplasmic reticulum is the cellular organelle responsible for the correct folding and sorting of eukaryotic proteins undergoing maturation [196]. A chronic failure of this maturation causes the accumulation of degradation-resistant misfolded proteins, resulting in ER stress. The adaptive mechanism that can resolve ER stress is termed the unfolded protein response (UPR) [197]. ER stress was initially identified as a central feature of insulin resistance in peripheral tissues [198], and later suggested as a possible link between overnutrition and hypothalamic leptin resistance [171]. The expression level of UPR signaling molecules such as PKR-like ER kinase (PERK), inositol-requiring-1 (IRE1), spliced form of X-box-binding protein-1 (XBP-1s), glucose-regulated/binding immunoglobulin protein-78 (GPR78) and C/EBP homology protein (CHOP) are increased in the hypothalamus of DIO models [171, 179, 183, 199, 200]. Hypothalamic UPR signaling is induced by the short-term ICV infusion of fatty acids [179]. In addition to DIO, ER stress is related to genetic human obesity caused by melanocortin-4 receptor (MC4R) gene variants. Obesity-associated MC4R variants are retained in the ER and cause ER stress, which results in impaired MC4R signaling and obesity. Pharmacological chaperone 4-phenyl butyric acid promotes the location of MC4R to the cell surface and subsequent MC4R signaling [201, 202].

Evidence for the contribution of ER stress to central leptin resistance comes from the finding that the prior ICV

injection of the ER stress inducer thapsigargin inhibits the activation of hypothalamic STAT3 and Akt by leptin and insulin [200]. Consistently, ER stress inducers suppress the leptin-induced phosphorylation of STAT3 in the ARC of hypothalamic slice preparations [203]. In addition, the central administration of chemical chaperones or ER stress inhibitors improves leptin sensitivity and alleviates weight gain in the DIO animal models [171, 199, 200].

HFD-induced hypothalamic ER stress may be caused by ectopic accumulation of lipids in the hypothalamic neurons (termed ‘lipotoxicity’) [204]. HFD induces the accumulation of palmitoyl- and stearoyl-CoA in the rat hypothalamus [172]. Treatment of hypothalamic neurons with palmitate causes ER stress which leads to reduced MC4R protein expression and a profound loss of MC4R signaling in response to anorexigenic melanocortin α -MSH [205]. As the hypothalamic melanocortin system is an important mediator of leptin actions, ER stress-induced impairment of hypothalamic melanocortin signaling may underlie the reduced hypothalamic response to leptin in animals on a palmitate-rich HFD.

Several mechanisms are suggested to bridge HFD-induced ER stress to impaired leptin receptor signaling. ER stress may lead to the activation of IKK β –NF κ B signaling in the hypothalamus [171]. Overnutrition has been shown to activate IKK β –NF κ B signaling in the hypothalamus through ER stress responses [171]. Conversely, induction of hypothalamic ER stress by HFD is abrogated by the inhibition of NF κ B signaling [206], which implies a positive feedback loop between NF κ B signaling and ER stress [171]. Peroxisomes are generated from the domain in the ER under increased metabolic pressure [207]. The peroxisome proliferator-activated receptor- γ (PPAR γ) is a nuclear receptor that senses the flux of increased lipids and regulates the transcription of genes involved in glucose and lipid metabolism [208]. PPAR γ agonist stimulates the formation of peroxisomes in hypothalamic neurons [209]. Hypothalamic activation of PPAR γ causes hyperphagia and weight gain. By contrast, a blockade of hypothalamic PPAR γ activity leads to a negative energy balance and improved leptin resistance in HFD-fed rats [209]. In POMC neurons, PPAR γ reduces the level of reactive oxygen species (ROS), that are a critical signal that stimulates POMC neuronal activity, via peroxisome proliferation and thus results in reduced neuronal activity [210, 211].

It was recently suggested that disruption of the mitochondrial-ER interaction may be a key element in leptin resistance in POMC neurons. Mitochondrial-ER contacts are decreased in the anorexigenic POMC neurons of DIO mice [212]. Furthermore, deletion of PPAR γ in POMC neurons enhances mitochondrial-ER interactions and increases leptin sensitivity in these neurons [211].

Mitofusin-2 is a mitochondrial transmembrane GTPase protein that mediates mitochondrial-ER contacts. Mice lacking mitofusin-2 in POMC neurons display a loss of these interactions, defective POMC processing, ER stress-induced leptin resistance, hyperphagia, reduced energy expenditure, and obesity. In these mice, the pharmacological reduction of hypothalamic ER stress rescues these metabolic defects. In contrast, the ablation of mitofusin-2 and mitofusin-1 in AgRP neurons disrupts mitochondrial fusion without inducing ER stress and alleviates HFD-induced obesity [213]. These findings indicate the importance of mitochondrial dynamics in AgRP neurons during the establishment of DIO.

On the other hand, certain UPS signaling pathways may have a protective role against overnutrition-induced leptin resistance. X-box binding protein 1 (XBP1) is an UPR transcription factor under the control of the IRE1 pathway and is processed to an active form by splicing upon ER stress [214]. The neuron-specific deletion of XBP1 increases the susceptibility to leptin resistance [199] whereas constitutive expression of XBP1 in POMC neurons improves leptin sensitivity by suppressing PTP1B and SOCS3 [203]. These results demonstrate the beneficial metabolic effects of XBP1 under conditions of overnutrition.

Miscellaneous

Several other signaling molecules have been shown to regulate leptin sensitivity in the hypothalamus. The JNK-signaling pathway plays a crucial role in the development of obesity and insulin resistance [215]. JNK1 disrupts insulin signaling via the serine phosphorylation of IRS-1 in peripheral organs [215]. JNK1 expression is elevated in the hypothalamus of DIO mice, which may result from lipotoxic stress and low grade inflammation [216, 217]. The neuronal ablation of JNK1 in mice prevents HFD-induced obesity and increases energy expenditure and insulin sensitivity, indicating a deleterious impact of neuronal JNK1 on leptin signaling and energy balance. These effects have been associated with the activation of a hypothalamo-pituitary-thyroid axis [216]. In line with this, the acute ICV injection of JNK inhibitor into *Lep^{ob}* mice rescues impaired hypothalamic insulin signaling and glucose intolerance [217]. Recently, it was also demonstrated that AgRP neuron-specific JNK activation increases the spontaneous firing of AgRP neurons and causes leptin resistance, hyperphagia and obesity [175].

A member of a novel class of PKC serine-threonine kinases, protein kinase- θ (PKC- θ), is activated by lipid metabolite diacylglycerol (DAG) and inhibits insulin-induced IRS1-tyrosine phosphorylation and PI3K activation in peripheral tissues [218]. PKC- θ is expressed in discrete

neuronal populations including the ARC NPY/AgRP neurons and the neurons of the hypothalamic DMH [219]. The systemic and central administration of palmitic acid increases the hypothalamic DAG level and promotes the membrane localization of PKC- θ in the hypothalamus, a marker of PKC- θ activation and of dampened hypothalamic insulin and leptin signaling. The knockdown of PKC- θ in hypothalamic ARC attenuates DIO and improves HFD-induced glucose intolerance. These results have suggested that the activation of hypothalamic PKC- θ may mediate the deleterious effects of a HFD on hypothalamic insulin and leptin signaling.

Elevation of the adenosine 3', 5'-monophosphate (cAMP) level impairs leptin-activated STAT3 and S6K signaling cascades via the induction of SOCS3 and PTP1B in organotypic hypothalamic slices [220]. This effect is mediated by Epac, a cAMP-regulated guanine nucleotide exchange factor for the small G protein Rap1 [221]. Hypothalamic Epac activation blunts the leptin-induced depolarization of hypothalamic POMC neurons and attenuates the anorectic responses to exogenous leptin. As hypothalamic Rap1 activity is enhanced in the hypothalamus of DIO mice, cAMP-Epac-Rap1 signaling may contribute to HFD-induced leptin resistance.

Closing remarks

In the last two decades since the discovery of leptin, numerous studies have demonstrated how this hormone functions in the hypothalamic neurons to maintain energy homeostasis. To explore signaling pathways that transduce the effects leptin in hypothalamic neurons, different research groups have used animal models lacking critical signaling molecules in these cells. The results obtained using this approach should be carefully interpreted however as deletion of essential signaling components in hypothalamic neurons may lead to an energy imbalance phenotype without affecting leptin signaling. It is also possible that abnormal metabolic phenotypes in genetically engineered animal models are the result of developmental defects in the neuronal circuits regulating energy balance.

Meanwhile, as obese humans and mice exhibit elevated levels of leptin in the systemic circulation and reduced responses to exogenous leptin, the molecular mechanisms underlying leptin resistance in hypothalamic neurons have been a major area of obesity research. To date, multiple negative regulatory pathways of leptin receptor signaling in the hypothalamus have been identified and are reviewed herein. However, recent studies using a leptin receptor antagonist have reported that endogenous leptin receptor signaling in the hypothalamus in DIO mice is comparable to lean mice, arguing against the concept of 'leptin

resistance' in DIO [88, 145]. Future studies will be required to address whether DIO mice have a reduced ability to respond to endogenous leptin and to reveal why obesity develops in subjects or animals with fully functioning leptin signaling pathways.

Several issues in hypothalamic leptin signaling pathways also need to be addressed in future studies. There is no doubt that LepRb is a primary receptor responsible for the metabolic and neuro-hormonal effects of leptin. However, it is possible that other receptors (for example, LRP proteins) or signaling molecules on the cellular surface may interact with leptin to evoke downstream signaling cascades that amplify the classical signaling cascades mediated through LepRb. Approaches to the discovery of novel receptors of leptin may help to develop therapeutic strategies to modulate leptin signaling. Recent evidence links classical leptin signaling to cilia signaling. Future studies are needed to elucidate where and how these signaling pathways are connected. Although POMC and AgRP neurons in the hypothalamic ARC are known as a major target of leptin, the deletion of LepRb in other neurons such as GABAergic neurons and neuronal nitric oxide synthase (NOS1)-expressing neurons recapitulates the obese phenotype seen in LepRb-null mice [222, 223]. Moreover, recent studies have shown that leptin acts on astrocytes to indirectly regulate hypothalamic neuronal activity [224, 225]. Identifying new cellular targets for hypothalamic leptin functions may greatly contribute to our understanding of the functional networks between hypothalamic neurons, or between neurons and glial cells or cells of the innate immune system within the hypothalamus, that control the energy balance.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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