Leptin-mediated ion channel regulation: PI3K pathways, physiological role, and therapeutic potential

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ABSTRACT

Leptin is produced by adipose tissue and identified as a “satiety signal,” informing the brain when the body has consumed enough food. Specific areas of the hypothalamus express leptin receptors (LEPRs) and are the primary site of leptin action for body weight regulation. In response to leptin, appetite is suppressed and energy expenditure allowed. Beside this hypothalamic action, leptin targets other brain areas in addition to neuroendocrine cells. LEPRs are expressed also in the hippocampus, neocortex, cerebellum, substantia nigra, pancreatic β-cells, and chromaffin cells of the adrenal gland. It is intriguing how leptin is able to activate different ionic conductances, thus affecting excitability, synaptic plasticity and neurotransmitter release, depending on the target cell. Most of the intracellular pathways activated by leptin and directed to ion channels involve PI3K, which in turn phosphorylates different downstream substrates, although parallel pathways involve AMPK and MAPK. In this review we will describe the effects of leptin on BK, KATP, Kv, CaV, TRPC, NMDAR and AMPAR channels and clarify the landscape of pathways involved. Given the ability of leptin to influence neuronal excitability and synaptic plasticity by modulating ion channels activity, we also provide a short overview of the growing potentiality of leptin as therapeutic agent for treating neurological disorders.

KEYWORDS
Ca2+ and TRP channels; cell firing; KATP and BK channels; leptin; NMDA and AMPA receptors; PI3K

The leptin receptor

Leptin, from the greek leptos (“thin”), is an adipokine first discovered nearly 20 years ago and proposed as a satiety signal that regulates food intake and energy balance.1 Leptin is a 16-kDa protein of 167 aminoacid residues encoded by the Ob gene. Produced by adipose tissue, leptin has been proposed for the treatment of obesity and diabetes but there is now increasing interest on its action on brain functions and neurological diseases.2 The primary site of action of leptin for body weight regulation is the hypothalamus. In this brain region the effects of leptin are integrated with those of other adipokines, gastrokines, and different signals to regulate energy homeostasis and body weight.3 In response to leptin, there is a reduction of the appetite and an increase in energy expenditure. Both, leptin deficiency (in ob-/ob- mice) and leptin resistance in the absence of its receptor (in db-/db- mice), lead to severe obesity, demonstrating that leptin acts as a key negative feedback signal to control food intake and body weight.4

Leptin receptors (LEPRs) are expressed in 6 alternative spliced variants, named LEPRa, LEPRb, LEPRc, LEPRd, LEPRe and LEPRF, all belonging to class I cytokine superfamily. Isoforms A, C, D and F are the short isoforms. They bind and transfer leptin from the periphery through the blood brain barrier. LEPRe is the soluble isoform that mediates the bioavailability of leptin, and LEPRb is the long isoform, primarily involved in leptin signaling.5 Leptin receptors have an extracellular domain of 840 aminoacids, a transmembrane domain of 34 aminoacids and different signals to regulate energy homeostasis and body weight.3 In response to leptin, there is a reduction of the appetite and an increase in energy expenditure. Both, leptin deficiency (in ob-/ob- mice) and leptin resistance in the absence of its receptor (in db-/db- mice), lead to severe obesity, demonstrating that leptin acts as a key negative feedback signal to control food intake and body weight.4
mammillary nuclei (PMV). Moderate expression is found in the periventricular hypothalamic nucleus and lateral hypothalamic area (LHA) and, at lower expression levels, in the paraventricular nucleus (PVH). For a recent detailed description of leptin action in the different hypothalamic nuclei see recent reviews by Flak and Meyers. Outside the hypothalamus, LEPRB mRNA is also present in numerous areas of the nervous system. High levels are reported in the thalamus, neo-cortex, Purkinje and granular cell layers of the cerebellum and hippocampus. LEPRB is also highly expressed in the chromaffin cells of the adrenal gland and pancreatic β-cells.

A combination of in vitro and in vivo studies over the past 10 years have led to a sharper understanding of how LEPRB regulates the intracellular signaling pathways of many cells. In the unbound state, LEPRB is a homodimer at the plasma membrane with effective docking sites for Janus kinases (JAK), a family of tyrosine kinases associated with intracellular cytokine signaling. Janus kinase 2 (JAK2) and SRC family kinases (SFKs) are constitutively associated with membrane-proximal regions of LEPRB. Following 1:1 binding of leptin to the receptor, the 2 subunits of the receptor undergo a conformational change leading to transphosphorylation and transactivation of JAK2 and SFK protein. JAK2, downstream, phosphorylates members of the signal transduction and transcription (STAT) family of intracellular proteins. STAT3 is so far the most widely used transcription factor activated by leptin, but there is also evidence for STAT3-independent pathways that could be activated specifically in pancreatic β-cells. Once activated, STAT3 translocates to the nucleus and stimulates transcription of target genes that mediate leptin’s action at the cell level (Fig. 1).

**PI3K and leptin: An effective duo for ion channel regulation**

PI3Ks are lipid kinases that phosphorylate phosphoinositides on position 3 of their inositol head group. The two main phosphoinositide products created in response to extracellular stimuli are PIP2 and PIP3. A number of studies have highlighted the relevance of PI3K in leptin-activated pathways. Hirsch and collaborators recently gave a complete overview of the different PI3K isoforms and their relationship to leptin and obesity. Focusing on its anorexigenic action, leptin regulates the transcription of different key neuropeptides. In the hypothalamic arcuate nucleus, leptin activates PI3K in 2 different populations of neurons, a first group expressing pro-opiomelanocortin (POMC) and a second one coexpressing neuropeptide Y and Agouti-Related Protein (NPY/AgRP) which regulate feeding behavior and body weight homeostasis. In particular, leptin regulation of food intake presents a cell type-specific neuron activation: it depolarizes POMC neurons while hyperpolarizes NPY/AgRP ones. More specifically, PI3K triggers the activation of phosphodiesterase3B (PDE3B), which is a prerequisite for leptin-stimulated STAT3 activation in NPY neurons. This clearly indicates that the PI3K–PDE3B–cAMP pathway is an important pathway of leptin signaling in the hypothalamus for feeding regulation.

PI3K signaling has emerged also as the main pathway through which leptin alters neuronal excitability and secretion by activating K<sub>ATP</sub>, BK and TRPC channels as indicated in Figure 1 (red pathways). In hippocampal neurons, leptin activates BK channels via PI3K-dependent reorganization of actin filaments and subsequent clustering of BK channels at synapses. PI3K has also a central role in K<sub>ATP</sub> channel activation in the arcuate nucleus of the hypothalamus. Here, cytoskeletal remodelling is a key step for the signaling mechanisms. In a mouse hypothalamic cell line, leptin induces actin filament depolymerisation via PI3K. Emerging evidence has suggested that PI3K pathways play also a critical role in the early steps of exocytosis. PI3K activation in this case induces F-actin depolymerisation by acting on cytoskeleton formation, with a similar mechanism leading to K<sub>ATP</sub> channel activation. More specifically, PI3K phosphorylates the C-terminal tail of PTEN (phosphatase and tensin homolog) in hypothalamic neurons and inhibits its phosphatase activity. The resulting PTEN inhibition, in turn, produces actin remodelling through F-actin depolymerisation (see Fig. 1).

**Leptin-mediated ion channel regulation**

The interest of leptin on ion channel regulation in central neurons and neuroendocrine cells started soon after its discovery in 1994. It was immediately evident that leptin is very effective in activating K<sub>ATP</sub> channels in pancreatic β-cells and central neurons. A finding soon extended to BK channels that are critical
for the control of neuronal firing and cell excitability. Presently, there is increasing interest in understanding the action and the landscape of pathways by which leptin acts on a multitude of ion channels, including: $K_{\text{ATP}}$, BK, $\text{Ca}^{\text{v}}$, TRPC, NMDAR, AMPAR and ENaC. The idea is to achieve a converging view that could account for the different effects of leptin on the many membrane channels that regulate cell activity at rest and during stimulation. Below we will give an overview of the past and recent papers reporting the effects of leptin on channels listed above, which regulate central neurons and neuroendocrine cells activity (see Table 1).

### $K_{\text{ATP}}$ channels

As a general mechanism of action on cell excitability, leptin exerts a hyperpolarizing effect on neurons and

| CHANNEL | CELL TYPE | EFFECT ON CHANNEL | EFFECT ON CELL | PATHWAY | REF |
|---------|-----------|-------------------|----------------|---------|-----|
| $K_{\text{ATP}}$ | Hypothalamic NPY neurons/Pancreatic $\beta$-cells | Channel opening via F-actin depolymerisation/Increased channel trafficking | Cell hyperpolarization/Increased HVA currents/Improved electrical remodelling | JAK/STAT PTEN AMPK | 12-15,17,27,30,33,36,38,39,41-46 |
| BK | Hippocampal neurons/Chromaffin cells | Increased channel open probability/Increased channel trafficking | Cell hyperpolarization and AP firing block at rest/Sustained AP firing and increased exocytosis during stimulation | JAK/STAT PI3K | 17,35,37,50,53-60 |
| NMDAR/AMPA | Hippocampal neurons/Substantia gelatinosa | Facilitation of NMDAR function | Increased synaptic plasticity (LTP, LTD)/Enhanced nociception | MAPK/ERK JAK/STAT PI3K | 67-69,70-76 |
| $\text{Ca}^{\text{v}}$ | Hypothalamic POMC neurons/Insulinoma cell lines | Increased expression of L-type $\alpha_{1}$-subunit | Increased HVA currents/Increased exocytosis/Increased dendritic spine number/POMC neurons depolarization | JAK/MAPK PI3K/IR/PI3K | 16,77-79,39-84 |
| TRPC | Hypothalamic neurons/POMC neurons/Insulinoma cell lines | Increased channels trafficking | Improved electrical remodelling/Cell hyperpolarization/Cell hyperpolarization | JAK/STAT MAPK PI3K/PI3K | 41,86 |
| $K_{\text{v}}$ | Pancreatic $\beta$-cells | Increased channel expression/Increased channel trafficking via actin depolymerisation | Improved electrical remodelling/Cell hyperpolarization/Cell hyperpolarization | JAK/STAT MAPK PI3K | 41,86 |
| ENaC | Endometrium | Downregulation of $\gamma$-ENaC expression | Attenuation of endometrial receptivity | JAK/STAT | 88 |
neuroendocrine cells that derives from \(K_{ATP}\) or BK channel activation. Convincing evidence has been found in hypothalamic neuropeptide Y neurons (NPY), hippocampal neurons, chromaffin cells of the adrenal gland and pancreatic \(\beta\) cells.

Focusing on the regulation of \(K_{ATP}\) channels, leptin exhibits distinct effects on hypothalamic orexigenic and anorexigenic neurons. Neurons coexpressing neuropeptide Y, Agouti-related peptide (AgRP) and GABA are orexigenic (named NAG) and regulate food intake. Separate populations of neurons, expressing the pro-opiomelanocortin peptide (POMC), are anorexigenic. Interestingly, both NAG and POMC neuronal activity is regulated by leptin: NAG neurons are inhibited through the activation of \(K_{ATP}\) channels via PI3K, while leptin signaling in POMC neurons leads to increased neuronal activity through TRPC channels activation, as described below. The block of action potential firing is due to a hyperpolarizing effect of leptin (4–50 nM) induced by \(K_{ATP}\) activation. A similar effect is shown in hypothalamic slices, where leptin decreases the firing rate of action potentials and input resistance.

Regulation of \(K_{ATP}\) channels by leptin is not restricted to the nervous system. Indeed, \(K_{ATP}\) channels are also involved in the control of pancreatic \(\beta\)-cells excitability. In this case, the phosphatase and tensin homolog (PTEN) plays a pivotal role in \(K_{ATP}\) channels activation. Leptin inhibits PTEN by increasing PI3K-mediated phosphorylation of the phosphatase. PTEN inhibition induces F-actin depolymerisation and consequently \(K^+\) channel opening (see Fig. 1). Furthermore, leptin is shown to promote \(K_{ATP}\) channels trafficking from cytosolic vesicles to the plasma membrane of \(\beta\)-cells through the stimulation of AMP-activated protein kinase (AMPK). Thus, in general, leptin hyperpolarizes both anorexigenic neurons and pancreatic \(\beta\)-cells through activation of \(K_{ATP}\) conductances.

**BK channels**

In mammalian central neurons, \(Ca^{2+}\)-activated \(K^+\) channels and voltage-activated \(K^+\) channels contribute to set the resting potential and shape neuronal firing. Large-conductance \(Ca^{2+}\)-activated potassium channels (BK) are widely expressed in dendrites, axons and synaptic terminals and, thus, they regulate neuronal signaling and neurotransmitter release. BK channels activate in response to calcium influx during action potentials and contribute to spike repolarization and fast after-hyperpolarization. They limit calcium entry and transmitter release by reducing the duration of presynaptic spikes at neurosecretory nerve terminals. In the hippocampus, BK channels act as an “emergency brake” that would control transmitter release only during excessive depolarization and accumulation of intracellular calcium. In this brain area, leptin hyperpolarises CA1 pyramidal neurons by activating postsynaptic BK channels, moving the membrane potential of these neurones away from their firing threshold. A recent multielectrode array (MEA) study on cultured hippocampal neurons shows that leptin decreases the spontaneous firing activity in both young (11 DIV) and aged (18 DIV) neurons grown in culture. The effect is reversed by paxilline, suggesting a key role of BK channels in the regulation of bursts firing patterns and timing of hippocampal microcircuits (Fig. 2). Although the \(K^+\) channel types targeted by leptin in the hippocampus and hypothalamus are different (BK in hippocampus and \(K_{ATP}\) in hypothalamus), the signaling pathway involved is common and mediated by PI3K.

As mentioned above, leptin receptors are expressed in the peripheral nervous system and neuroendocrine cells. Chromaffin cells of the adrenal medulla express high densities of \(LEPRB\) and are thus an important target of leptin action that accounts for a role of the hormone in the regulation of circulating catecholamine under basal conditions or during sustained sympathetic stimulation. More specifically, leptin exhibits marked effects on mouse chromaffin cell (MCCs) excitability, which derives from an effective potentiation of BK channel activation. As BK channels together with SK and Cav1.3 channels set the firing rate and degree of spike adaptation of MCCs at rest and during sustained stimulation, it is not surprising that strong activation of BK by leptin could lead to drastic changes of AP firing. As reported by Gavello et al., leptin exhibits a dual action on MCC activity. At rest, the adipokine inhibits the spontaneous firing of most cells by increasing the probability of BK channel opening through a PI3K-signaling cascade that hyperpolarizes the cell and stops the firing (Fig. 3a). On the contrary, during sustained stimulation, leptin preserves cell excitability by generating well-adapted AP trains of lower frequency and broader duration. This, together with the observation that
leptin increases catecholamine secretion by increasing the size of the ready-releasable pool and the rate of vesicle release, highlighting the importance of the adipoadrenal axis in the leptin-mediated increase of sympathetic tone and catecholamine synthesis and release.\(^{35,61,62}\)

Of great interest is the molecular mechanism by which leptin potentiates BK channel activation via PI3K. Detailed measurements on single BK channels in “perforated vesicles” (left inset in Fig. 3b) and macroscopic currents in the “perforated patch” configuration show that leptin specifically increases BK channel open probability without altering the unitary channel conductance (Fig. 3b). Leptin causes also a 12 mV leftward shift (ΔV) to the voltage-dependence of BK channel conductance (\(g_{\text{BK}}(V)\)). This enhances the probability of BK channel opening at lower voltages (Fig. 3c) and explains the potent hyperpolarizing action of leptin on chromaffin cell excitability at rest. Leptin-driven hyperpolarization is fully prevented by paxilline, a specific BK channel blocker,\(^{63}\) and by wortmannin, a specific PI3K blocker\(^{64}\) (see ref.\(^{27}\) for details). It is interesting that similar effects of leptin are reported in the hippocampus\(^{17,65}\) where BK channels control the rate of firing and the degree of action potential adaptation, suggesting common mechanism of leptin action in different excitable tissues.

BK channel activity is regulated either directly or indirectly through a wide range of modulatory pathways. Among the direct regulatory mechanisms, there is the addition of a phosphate group to functionally important residues (Ser/Thr/Tyr) of the channel pore forming α subunit. The reactions are catalyzed by PKA, PKG and PKC with a phosphorylation-dependent effect that can be either stimulatory or inhibitory depending on the channel open probability.\(^{66}\) Little is known on the mechanism by which PI3K alter BK channel activation. It is hypothesized that also PI3K, as other kinases, activates BK channels by direct phosphorylation of a serine residue, but further studies are needed to clarify this issue.

**NMDA and AMPA receptors**

Besides affecting hippocampal excitability, leptin has a prominent role in synaptic plasticity. It facilitates the development of long-term potentiation (LTP) in different brain areas.\(^{67,68}\) This effect is associated with enhanced NMDAR synaptic transmission, with a consequent NMDAR-induced intracellular calcium increase.\(^{69}\) Moult & Harvey (2011)\(^{70,71}\) reported that 25 nM leptin induces NMDAR-dependent hippocampal plasticity through an age-dependent mechanism.\(^{72}\) Depending on the developmental stage of neurons,
leptin can induce either long lasting depression (LTD) in rat hippocampal slices from P5-8 or transient synaptic depression in P11-18 animals. On the contrary, leptin induces a persistent increase in synaptic strength (LTP) in adult slices (12–16 week and 12–14 month). Both, long lasting (P5-8) and transient (P11-18) synaptic depressions are mediated by a MAPK/ERK signaling pathway, but not PI3K (green pathway in Fig. 1). Conversely, the persistent increase of excitatory synaptic transmission in adult slices is mediated by PI3K and requires the activation of GluN2A, but not GluN2B subunits. Moreover, in rat juvenile hippocampus leptin induces LTP in excitatory synaptic transmission at the temporoammonic (TA) input to CA1 pyramidal neuron synapses (TA-CA1). This effect is NMDAR-dependent and

Figure 3. (A) Representative current-clamp recording of the spontaneous electrical activity of a mouse chromaffin cell before, during and after leptin application (1 nM). At the right are shown 2 overlapped action potentials (APs) at an expanded time scale in order to compare AP waveforms in control condition (black) versus leptin treated condition (red). The asterisks indicate the AP enlarged in the inset. (B) Single channel experiments in perforated microvesicle recording conditions, as illustrated to the top left of the panel. Recordings were in control conditions and in presence of 1 nM leptin or 1 μM paxilline. As shown to the right, leptin increases the open probability of BK channels (red trace). The single channel activity remaining after blocking BK channels with paxilline is due to SK channels (bottom trace) (see54). (C) Shown at the left are the BK channel conductance (gBK) in control conditions (black curve) and in presence of 1 nM leptin (red curve). gBK was calculated as Ipeak/(V – Erev). Data were fit by a Boltzmann equation with mean V1/2 = –34.2 mV (control) and V1/2 = –21.9 mV (leptin). Shown at the right are voltage-clamp representative recordings of the BK current in control condition (black trace) and in presence of leptin (red trace). Below is the protocol used: a 50 ms Ca2+ -loading step at 0 mV followed by a 400 ms test step at +120 mV and then back to the holding potential (–70 mV). Adapted from.
involves the activation of GluN2B subunits. It is known that leptin alters neuronal/synaptic function and structure, and influences neuronal survival and proliferation in the hippocampus, amygdala, cerebellum, brainstem and substantia nigra. Overall, these effects lead to a leptin-driven improvement of learning, memory and other forms of cognition and resistance to insults that impairs cognitive performance.26

Leptin plays also a pivotal role in nociceptive sensing in animals with peripheral nerve injury. The adipokine enhances spinal NMDAR-induced currents in substantia gelatinosa (SG) neurons through a JAK2/STAT3 signaling pathway. There is evidence that repeated exposures to leptin induce NMDAR-mediated thermal hyperalgesia and mechanical allodynia similar to the one induced by peripheral nerve injury. Thus, there is a tight relationship between the spinal leptin effect and NMDAR-mediated neuronal excitation and nociceptive signaling, supporting an important role of the adipokine in nociception. Moreover, leptin can also regulate AMPA receptors. In particular, leptin inhibits AMPAR-mediated synaptic transmission pre- and post-synaptically through a PI3K-mediated pathway in mouse hippocampal slices.76

Voltage-gated Ca\(^{2+}\) channels

Voltage-gated Ca\(^{2+}\) channels (VGCCs) are also key targets of leptin in hypothalamic neurons and chromaffin cells. In particular, leptin promotes the expression of functional L-type Ca\(^{2+}\) channels (Cav1), which results in increased hormone release in gonadotropes, through the amplification of the stimulatory actions of GnRH.77 48 hours application of 30 nM leptin on clonal gonadotrope LβT2 cells induces a significant increase of Cav1 currents that is likely associated with Cav1.3 channels since quantitative RT-PCR reveals increased mRNA of this L-type α1 subunit.77 Leptin action on Ca\(^{2+}\) channels is evident also in NPY neurons of the ARC nucleus of hypothalamus. Here, leptin decreases the peak amplitude of high voltage-activated (HVA) calcium currents. The pathway involves JAK2 and MAPK. In contrast, leptin increases the amplitude of HVA currents in POMC-containing neurons through a PI3K-mediated pathway. Thus, leptin affects Cav1 channels in both hypothalamic neuron types with opposing effects. Interestingly, leptin action on these neurons is rapid and partially reversed, suggesting direct modulation of

L-type channel activity rather than altered gene expression mediated by JAK2.78 Moreover, leptin regulates L- and N-type channels following JAK2 activation in cultured porcine chromaffin cells. Activation of JAK-STAT pathway leads also to increased levels of TH-mRNA through the MAPK pathway.79 Quite different results are obtained in MCCs. In these cells, 30 min incubation with 1 nM leptin do not alter Ca\(^{2+}\) current densities, although a significant increase in catecholamine secretion is observed.35 in line with previous findings by Takekoshi et al.16,79.

TRPC channels

Leptin is also reported to exhibit a neurotrophic action that leads to synapse formation and remodelling in both the hypothalamus and the hippocampus.80-84 The effect is more evident in the hippocampus, where leptin positively influences cognition, anxiety and depression that are critically dependent on dendritic spine number and shape. In this brain region, leptin induces the formation of dendritic protrusions (thin headless, stubby and mushroom shaped spines), by trafficking and activation of TRPC channels in cultured hippocampal neurons. Leptin-activation of TRPC currents is dose-dependent and prevented by the targeted knockdown of the leptin receptor LEPRB. The mechanism by which leptin increases TRPC trafficking to the membrane is a CaMK/βPix-dependent pathway.80 The leptin-induced TRPC4 activation is essential for AMPK activation and thus for the further regulation of K\(_{ATP}\) channels trafficking in insulinoma cell lines33 (Fig. 1). In addition, as previously mentioned, leptin depolarizes POMC hypothalamic orexinergic neurons. This effect is attributed to TRPC4 and TRPC5 channels activation since the cationic inward current is potentiated over 2 fold by intracellular Ca\(^{2+}\) and extracellular La\(^{3+}\).39 As La\(^{3+}\) is a potent blocker of TRP1, TRP3, TRP6 and TRP7 channels85 these observations exclude the involvement of these TRPC channel isoforms. POMC neurons express TRPC channel transcripts with TRPC5 being the most prevalent. Thus, the proposed mechanism for TRPC4 and 5 channel activation following leptin binding to its receptor, includes JAK2 activation, IRS proteins phosphorylation and PI3K activation. Downstream, PI3K subsequently activates PLC\(_{γ}\)1, thus enhancing TRPC4 and 5 currents and POMC neuronal excitability.39 The net effects of TRPC channels activation in this
specific group of neurons is an increase of firing frequency and consequent release of anorexigenic peptides, which leads to the reduction of appetite as typically occurs during leptin action.37

**K⁺ channels, ENaC channels**

Other ionic conductances are involved in leptin physiology apart from the nervous system. The stimulation of cardiac leptin receptors is associated with JAK2/STAT3 signaling and with the activation of MAPK and PI3K pathways. All pathways are implicated in myocyte hypertrophy and cardioprotection. Enhanced PI3K signaling has been associated with up-regulation of K⁺ currents and improvement of electrical remodelling. This has been observed in adult rat ventricular myocytes, where chronic exposure to leptin increases both the expression and function of transient outward K⁺ currents, via upregulation of Kv4.2 and Kv4.3 in an AKT-dependent manner86 (Fig. 1). Similarly, leptin causes a transient increase of Kv2.1 channel trafficking in rodent and human β-cells, through the activation of PKA. The increased Kv2.1 surface expression by leptin is due to F-actin depolymerisation as previously shown for K<sub>ATP</sub> channels in the same cellular model41 (Fig. 1). Thus, altogether leptin induces hyperpolarization and block AP firing, through either K<sub>ATP</sub> or Kv2.1 channels.

The relationship between leptin and voltage-gated Na⁺ channels (Nav) has received little attention so far. To the best of our knowledge, at present there is no evidence suggesting any effect of leptin on Nav channels. On the contrary, it has been shown that the interaction between leptin may reduce endometrial receptivity by activating the STAT3 signal pathway and down-regulating γ-ENaC expression in the endometrium and, in general, in epithelial tissues.87 An abnormal ENaC gene expression appears to be associated with early pregnancy loss. Thus, high leptin attainmate endometrial receptivity and increase very early pregnancy loss by down-regulating γ-ENaC channel expression.88

**Leptin-based therapeutic strategies for treating NS disorders**

Besides regulating energy balance and body weight by acting on hypothalamic nuclei,3,8 leptin action appears more widespread than expected, involving many brain regulatory targets. As discussed above, leptin alters synaptic plasticity and neuronal excitability by activating NMDA,14,69,70 BK,73,53 and K<sub>ATP</sub> channels,14 with a surprising ability to counteract brain hypoxic conditions, epilepsy and depression.17,53,81,89 Leptin also regulates calcium entry into the cell through Cav and TRPC channels activation and it is thus able to regulate key cellular mechanisms such as cell excitability and neurotransmitter release.39,77,78,80 These new findings may lead to possible therapeutic usage of leptin in treating a number of neurological and neuropsychiatric disorders. Among the many proposed, we list below the more promising for future therapeutic approaches.

**Leptin effects on neuroprotection**

Given the increasing incidence of neurodegenerative disorders on social life, the search of new neuroprotectants to prevent brain damage is rapidly growing.90-93 Most neuroprotective compounds have failed in clinical trials because of dose-limiting side effects. Conventional neuroprotectants target specific classes of neurotransmitter receptors that are involved in the neurotoxic cascade, such as the NMDA receptor.94 Moreover, accumulation of pathologic levels of intracellular Ca<sup>2+</sup> is a major proximal cause of cell death during ischemia. Ca<sup>2+</sup>-channel antagonists (isradipine, nifedipine, amiodipine) can only partially prevent Ca<sup>2+</sup> entry when all voltage-gated Ca<sup>2+</sup> channels (L, N, P/Q, R, T) are available and have significant side effects, like hypotension, as they target cardiac and vascular muscle tissues. Targeting BK potassium channels by increasing their open probability seems a promising alternative strategy in this context.95 BK channels react to rapid Ca<sup>2+</sup> increases and membrane depolarization by repolarizing the cells and damping further voltage-dependent Ca<sup>2+</sup> influx. Pharmacological blockade of BK channels has modest effects on neuronal function,96 while their pharmacological activation could effectively regulate AP waveforms and AP firing. Thus, the use of potent, effective and specific BK channel openers would limit Ca<sup>2+</sup> entry during prolonged cell depolarizations, producing targeted neuroprotection in experimental stroke and brain hypoxic conditions.95 In this regard, evidence has accumulated supporting the idea that leptin might exert neuroprotective effects in various neurological disease models. In *in vitro* neurons, leptin attenuates cell death induced by removal of serum or neurotrophins,97,98 improves cell survival in models of ischemic stroke81 and protects against glutamatergic excitotoxicity and oxidative stress.97 Moreover, leptin has been
shown to counteract the hypoxia-induced inhibition of hippocampal microcircuits spontaneous activity through BK channel activation. Thus, again, BK channels play a central role in protecting neurons from damage related to cytosolic calcium dysregulation. Hypoxia is considered also a key risk factor for the onset of neurodegenerative disorders such as Alzheimer disease (AD). In this case, counteracting brain hypoxia could help overcoming AD and neurodegeneration. Indeed, given its ability to counteract β-amyloid induced damage, leptin is now considered a new potential therapeutic agent against AD.

The powerful neuroprotective effect of leptin is also evident in cortical neurons. Here, BK channel activation following increases in intracellular Ca2+ is a key step for leptin-induced neuroprotection in NMDA-exposed cortical neurons in vitro, limiting cell damage during excitotoxicity. Altogether, these findings demonstrate the great potentiality of leptin-based intervention via the PI3K/BK channel activation as a pathway to counteract neurodegenerative diseases.

Leptin as anticonvulsant, analgesic and antidepressant: effects on AMPARs and NMDARs

Besides being proposed as neuroprotectant, leptin is suggested also as effective anti-epileptic for its ability to counteract epilepsy in different rodent models, uncovering a possible therapeutic potential for the treatment of seizures. Indeed, focal seizures induced by neocortical injections of 4-aminopyridine (4AP) in rats are shortened and reduced in number by co-injection of leptin. In addition, leptin alone reduces neuronal spiking in an in vitro seizure model. The mechanism proposed for this leptin-driven anti-epileptic activity is associated with the inhibition of AMPAR-regulated synaptic transmission. The effect is JAK2/PI3K-mediated and displays a U-shaped dose response, suggesting that the main action of AMPAR inhibition at low doses may be paralleled by other mechanisms such as the ability of leptin to activate BK channels, hyperpolarizing the cells and thus reducing excitability. That leptin has clear anti-convulsant properties is demonstrated also by the ability to reduce hippocampal excitability in 2 distinct epilepsy mouse models.

Leptin has also an effective action on nociception. An increasing number of studies indicates that leptin enhances NMDAR-mediated synaptic transmission by recruiting an increased density of functioning NMDAR through a JAK2/STAT3 pathway. A consequence of this is that leptin superfusion on spinal cord slices dose-dependently enhances NMDAR-mediated currents in spinal cord lamina II neurons, while repeated intrathecal administration of leptin in young rats elicits thermal hyperalgesia and mechanical allodynia. The interaction between leptin’s spinal effects and NMDAR function supports a critical role for leptin in the pathogenesis of neuropathic pain and suggests a possible strategy of pharmacological interventions of neuropathic pain by blocking the effect of leptin and its intracellular signaling pathways.

Leptin is also proposed as regulator of mood and emotions and, as such, has been recently proposed as a potential novel antidepressant. Interestingly, the plasma levels of leptin in rats exposed to chronic unpredictable tests (tail suspension tests or forced swimming tests) is low. Systemic leptin treatment increases the leptin deficit and dose-dependently improves rats “behavioral despair” during forced swimming tests. The same effects occur by infusing leptin directly in the hippocampus, suggesting that hippocampus is the brain site that mediates leptin’s antidepressant-like effects. Focusing on the cellular and molecular mechanism Wang et al. have recently shown that leptin effects originate at the dentate gyrus (DG)-CA3 hippocampal region, where abnormal development and dysfunction of glutamergic synaptic transmission contribute to the pathogenesis of depression and other psychiatric disorders. Leptin treatment indeed dampens the increased glutamate release and the associated NMDA transmission evoked by forced swimming tests or tail suspension tests in DG-CA3, suggesting novel therapeutic strategies for the treatment of psychiatric disorders based on leptin.

Concluding remarks

A special issue of Metabolism in 2015, dedicated to the 20 years of leptin discovery, overviews the impressive progress made in these last 2 decades to understand the broad spectrum of functions covered by leptin as anti-obesity hormone, metabolism regulator, neuroprotective agent, and antidepressant. The great interest in leptin and its actions has grown in parallel to the discovery of leptin receptors in many different tissues, suggesting specific mechanisms controlled by this protein. Here, we reviewed the multiple
relationships between leptin, ion channels and PI3K, pointing the attention on the intriguing ability of leptin to activate different ionic conductances that converge on the regulation of resting neuronal conditions, cell excitability and neurotransmitter release. Some of the pathways activate Kv, KATP and BK channels that hyperpolarize resting neurons and give rise to the anti-apoptotic and neuroprotective effects of leptin, justifying the therapeutic potential of the adipokine for the treatment of brain injury and neurodegenerative disorders. Other pathways lead to the activation of NMDARs with consequent increased excitatory synaptic responses that alter behavior and cognition, as in the case of reduced states of depression or improved reward processing mediated by midbrain dopaminergic neurons. Much remains to be done to understand the molecular details of ion channel modulation by PI3K, AMPK and MAPK/ERK pathways. Solving them will be certainly beneficial for better understanding the crucial role of a complex hormone like leptin that acts at the intersection of neuroendocrinology, metabolism and brain disorders.

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