The PI3K signaling pathway mediates the biological effects of leptin

A via de sinalização intracelular da PI3K medeia os efeitos biológicos da leptina

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SUMMARY

The activation of the leptin receptor recruits several intracellular signaling pathways, including the phosphatidylinositol 3-kinase (PI3K) pathway. While some of the leptin-induced signaling pathways, such as the JAK2/STAT3 pathway, induce cellular responses primarily through changes in gene expression, the PI3K pathway affects cellular properties more rapidly, through post-translational changes such as protein phosphorylation. Accordingly, several studies have shown that the PI3K pathway is required for the acute effects of leptin, such as a leptin-induced decrease in food intake. Leptin signaling through PI3K also affects the electrophysiological properties of neurons, including changes in their membrane potential and firing rates. In this review, we summarize the recent advances in our understanding of the role played by the PI3K signaling pathway in controlling food intake and energy balance. In particular, we focus on the importance of the PI3K signaling pathway as a mediator of the effects of leptin on hypothalamic neurons. Arq Bras Endocrinol Metab. 2010;54(7):591-602

Keywords

Phosphatidylinositol; phosphatidylinositol 3-kinases; energy balance; insulin; Akt; hypothalamus

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SUMÁRIO

A ativação do receptor de leptina recruta diversas vias de sinalização intracelular, entre elas a via da fosfatidilinositol 3-quinase (PI3K). Enquanto algumas dessas vias, como a sinalização pelo JAK2/STAT3, induzem respostas celulares por meio de mudanças na transcrição gênica, a via da PI3K altera propriedades celulares de forma rápida, via fosforilação de proteínas. Em concordância, estudos mostraram que a via da PI3K é necessária para que a leptina induza seus efeitos agudos, como redução da ingestão alimentar, após administração de leptina. A ativação da PI3K pela leptina também afeta as propriedades fisiológicas de neurônios, incluindo mudanças no potencial de membrana e no potencial de ação. Nesta revisão, resumimos os recentes avanços na compreensão do papel desempenhado pela via de sinalização da PI3K no controle da ingestão alimentar e do balanço energético. Discutimos, principalmente, como a via da PI3K é importante para mediar os efeitos da leptina sobre os neurônios hipotalâmicos. Arq Bras Endocrinol Metab. 2010;54(7):591-602

Descritores

Fosfatidilinositol; fosfatidilinositol 3-quinase; balanço energético; insulina; Akt; hipotálamo

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INTRODUCTION

Phosphatidylinositol 3-kinases (PI3Ks) are heterodimeric complexes composed of regulatory and catalytic subunits that recruit lipids as second messengers and control a wide variety of cellular functions, such as survival, growth, metabolism, and chemotaxis. The diverse biological functions exerted by the PI3K signaling pathway have attracted attention because of the therapeutic potential of PI3K-targeted drugs. Pharmacological compounds that target specific PI3K subunits might be used for the prevention and/or treatment of inflammation and autoimmune diseases, hypertension, thrombosis, cardiac dysfunctions, and cancer (1-3).

The PI3K complex phosphorylates the 3-hydroxyl group of the inositol ring in lipid substrates known as phosphatidylinositols (PtdIns). The activity of PI3Ks is counterbalanced by phosphatase enzymes that specifically remove the phosphate residues from the PtdIns. One example is the phosphatase and tensin homolog, deleted on chromosome ten (PTEN), which dephosphorylates the 3-hydroxyl group of the inositol ring (Figure 1).

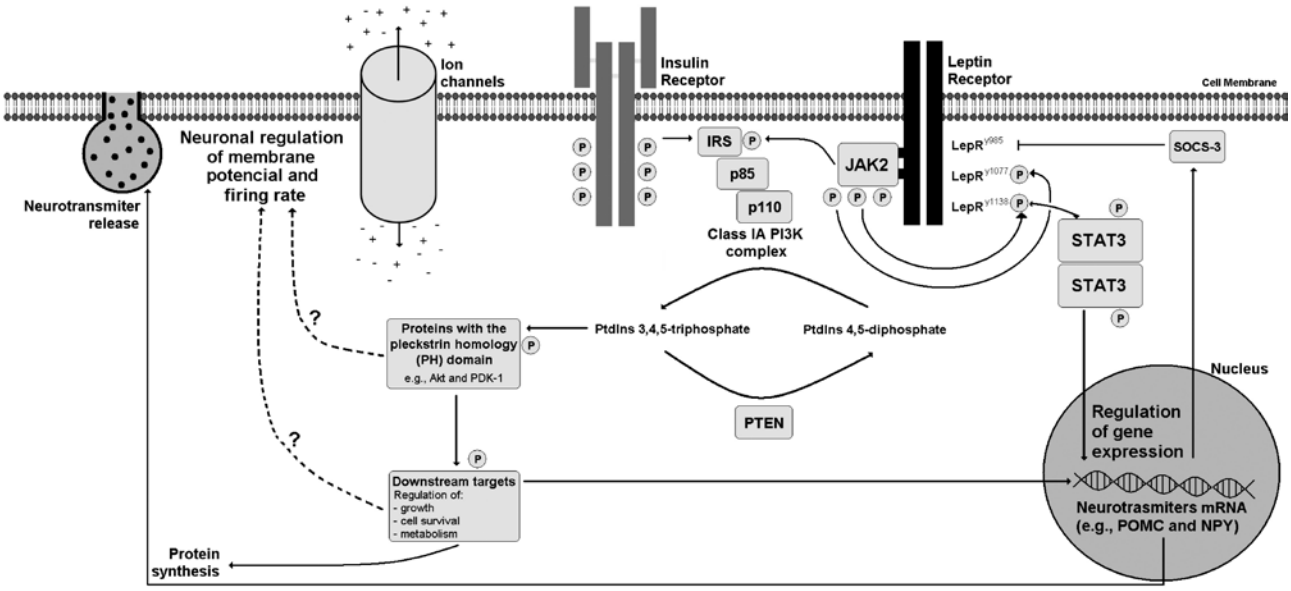

Figure 1. Scheme that illustrates intracellular signaling pathways recruited by leptin, particularly the PI3K pathway. Note that activation of leptin receptor (LepR) induces the subsequent phosphorylation and recruitment of Janus tyrosine kinase -2 (JAK2). JAK2 phosphorylates the LepR^{y1077} and LepR^{y1138} residues, and the insulin receptor substrates (IRS). The phosphorylation of LepR^{y1138} residue will recruit the signal transducer and activator of transcription -3 (STAT3) that under phosphorylation it dimerizes and is transported to the nucleus, controlling the transcription of target genes, including the suppressor of cytokine signaling-3 (SOCS-3). By its turn, SOCS-3 binds to the LepR^{y985} residue, inhibiting the LepR signaling. The insulin receptor also phosphorylates the IRS, which binds to the p85 regulatory subunits of Class IA PI3K, inducing the action of the PI3K complex, also composed of p110 catalytic subunits (α, β or δ). When activated, the Class IA PI3Ks phosphorylates the phosphatidylinositol (PtdIns) 4,5-diphosphate in the 3-hydroxyl group of the inositol ring, generating PtdIns 3,4,5-triphosphate. The activity of PI3Ks is counterbalanced by the enzyme phosphatase and tensin homologue (PTEN). Proteins that contain the pleckstrin homology (PH) domain are regulated by PtdIns 3,4,5-triphosphate and they are responsible to coordinate and convey the PI3K-dependent intracellular effects induced by leptin and insulin. These effects include the regulation of growth, cell survival, metabolism and chemotaxis through downstream targets. Regarding the effects of PI3K signaling in the regulation of energy balance, the induction of PI3K can affect the electrophysiological property of leptin-sensitive neurons, e.g., the membrane potential and fire rate, influencing the release of neurotransmitters. Besides, gene expression and protein synthesis are also regulated by downstream components recruited by PI3K signaling pathway. Interestingly, the mechanisms by which PI3K regulates ion channels properties are still unknown (dotted arrows).

The PI3K signaling pathway is induced by several growth factors and other hormones. For instance, activation of insulin receptor causes phosphorylation of tyrosine residues in insulin receptor substrate -1 and -2 (IRS-1 and IRS-2). This phosphorylation induces a rapid association between IRS-1 or IRS-2 and PI3Ks, causing the subsequent activation of the PI3K complex (4). The PI3K-induced phosphorylation of PtdIns activates downstream targets, such as Akt (also known as protein kinase B or PKB) and phosphoinositide-dependent kinase-1 (PDK-1), which, in turn, convey and coordinate most of the intracellular effects induced by insulin.

More recently, the PI3K signaling pathway was shown to play a role in the regulation of food intake and energy balance through hypothalamic neurons. Resembling many peripheral cells, neurons also respond to hormonal signals through the activation of intracellular pathways that regulate membrane potential, firing rate, the synthesis and release of neurotransmitters, and gene expression. Insulin and leptin are important hormones that act in specific populations of hypothalamic neurons, which ultimately control glucose homeostasis, food intake, energy expenditure and sympathetic activity.

In this review, we summarize recent advances in the understanding of the role of the PI3K signaling pathway in the control of food intake and energy balance. In particular, we focus on the importance of the PI3K pathway as a mediator of leptin's effects in hypothalamic neurons.

LEPTIN AND ENERGY BALANCE

The adipocyte-derived hormone, leptin, is a key component that controls body weight and energy homeostasis (5-7). Loss-of-function mutations in the genes that encode leptin or the leptin receptor (LepR) cause

hyperphagic obesity, *diabetes mellitus*, and neuroendocrine dysfunctions in rodents and humans (7,8). These monogenic mutations were initially described in mice (*ob/ob,* mutation in the leptin gene and *db/db,* mutation in the LepR gene) and were later shown to be recapitulated in humans. Importantly, leptin treatment in leptin-deficient humans or mice reduces food intake and body weight and restores their metabolic and neuroendocrine functions (9-11).

Innumerous studies have shown that the hypothalamus is the primary target of leptin in regulating energy balance, glucose homeostasis, and neuroendocrine function (12). The availability of leptin favors a reduction in food intake and body weight. On the other hand, fasting, a condition of low leptin levels, causes an opposite effect that can be partially or completely reverted by leptin replacement (5,6,13). For example, mice fasted for 48 h show increased expression of the orexigenic neuropeptide Y (NPY) and a reduction in the expression of pro-opiomelanocortin (POMC), which is a precursor of the anorectic peptide alpha melanocyte-stimulating hormone (α-MSH). However, intraperitoneal (i.p.) treatment of leptin during fasting prevents the changes in the expression of POMC and NPY in the arcuate nucleus (ARH), an important hypothalamic area affected by leptin (13,14).

Alternative splicing of the LepR mRNA and/or post-translational processing generates at least five isoforms of LepR (LepRa-e). Of the isoforms, LepRb (ObRb or long isoform) is considered the functional isoform, as upon leptin binding it induces a series of intracellular signaling cascades (15,16). LepRe does not carry a transmembrane domain and circulates as a leptin binding protein. The isoforms LepRa, LepRc, and LepRd have a short intracellular domain, compared to the LepRb isoform. As a consequence of incomplete intracellular domains, LepRa, LepRc, and LepRd are unable to activate intracellular pathways normally recruited by LepRb signaling (17). Because the focus of the present review is to discuss intracellular pathways recruited by leptin signaling, we will define LepRb as LepR.

LepR is a member of the class I cytokine receptors. Upon leptin binding, LepR undergoes dimerization that culminates in a cascade of intracellular events. In most cases, this cascade is triggered by the phosphorylation of a Janus tyrosine kinase (JAK) family member, known as JAK2. JAK2 is constitutively attached to LepR and becomes phosphorylated and activated when leptin binds to its receptor (17). Three tyrosine resides in the intracellular domain of LepR, named $LepR^{y985}$, $LepR^{y1077}$, and $LepR^{y1138}$, can become phosphorylated after leptin signaling. Phosphorylation of $LepR^{y985}$ reduces LepR signaling; therefore, this residue is considered a site for negative feedback. On the other hand, the phosphorylation of the $LepR^{y1077}$ and $LepR^{y1138}$ residues is necessary for the subsequent phosphorylation and recruitment of signal transducers and activators of transcription 5 and 3 (STAT5 and STAT3, respectively) (17).

The best-described signaling pathway recruited by leptin involves the coordinated activation of JAK2/STAT3. STAT3 is a transcriptional factor that, upon phosphorylation, dimerizes and is transported to the nucleus, where it controls the transcription of target genes (Figure 1). The phosphorylated form of STAT3 (pSTAT3) has been used as a marker of leptin-responsive neurons, whether the cells are activated or inhibited by leptin (18-20). One of the genes rapidly induced by leptin and regulated by pSTAT3 is the suppressor of cytokine signaling-3 (SOCS-3) (21). SOCS-3 is a family member of cytokine-inducible signaling inhibitors. It causes inhibition of LepR signaling by binding to $LepR^{y985}$ and blocking JAK2 activity (Figure 1). Changes in SOCS-3 expression have been related to conditions of leptin resistance, like those observed during diet-induced obesity. Accordingly, the hypothalamic expression of SOCS-3 is increased in obese animals (21). Besides, the genetic deletion of SOCS-3 in specific populations of hypothalamic neurons increases the leptin sensitivity and reduces the susceptibility to diet-induced obesity (22).

To directly test the importance of the LepR/JAK2/STAT3 signaling pathway on the biological effects of leptin, Bates and cols. (18) generated genetically modified mice in which the $LepR^{y1138}$ was substituted with a serine residue, blocking the leptin-induced phosphorylation of STAT3. Using this mouse model, the authors observed that leptin-induced STAT3 signaling is required for the long-term regulation of food intake, body weight and glucose homeostasis, as mice carrying this mutation became hyperphagic, obese, and diabetic (18). Nonetheless, these metabolic abnormalities were not as severe as those observed in *db/db* mice. Additionally, although *ob/ob* and *db/db* mice are virtually infertile, the mutant mice show a partial degree of fertility, suggesting that other signaling pathways are necessary for the entire biological effects of leptin.

In vitro studies also demonstrated that fast responses, presumably too rapid to be mediated by changes in gene expression, are evoked by acute application of leptin in energy homeostasis-related neurons (23-26). For instance, Spanswick and cols. (24) demonstrated that leptin applied to hypothalamic slices decreases the firing rate of action potentials and the input resistance of glucose-responsive neurons located in the ARH and in the ventromedial nucleus of the hypothalamus (VMH). These effects were accompanied by a slow progressive hyperpolarization to a new equilibrium, 5 to 15 min after application. Importantly, these effects were observed in wild-type animal models, but not in the obese Zucker rats, which have a mutant LepR isoform. The activation of ATP-sensitive potassium channel (KATP) was evidenced by the application of tolbutamide, a potassium channel blocker, which reversed the actions of leptin (24). Later, electrophysiological studies demonstrated that leptin mainly depolarized POMC neurons in 2 to 10 min, in a dose-response manner (27). The depolarization of POMC neurons was caused by a small inward current that reversed at about -20 mV, suggesting the involvement of a nonspecific cation channel. In addition, leptin treatment decreased the frequency of GABA-mediated inhibitory postsynaptic currents by 25% in 30% of the POMC cells, indicating that it may act presynaptically to reduce GABA release, which was confirmed by the application of tetrodotoxin (27). Therefore, these results indirectly imply that other intracellular pathways faster than JAK2/STAT3 signaling pathway, which requires changes in gene expression to convey its signal, are responsible for the acute leptin-induced changes in neuronal activity.

PI3K SIGNALING IS REQUIRED FOR THE ACUTE EFFECTS OF LEPTIN IN FOOD INTAKE

Initial reports, showing that the activity of the PI3K signaling pathway in the brain is important for the regulation of food intake, employed pharmacologic inhibitors of PI3K, including wortmannin and LY294002. In a classic study, wortmannin or LY294002 was administered intracerebroventricularly (i.c.v.) to Wistar rats (28). These authors observed that i.c.v. injection of LY294002 did not change food intake or body weight after 4 h or 24 h of treatment. However, pretreatment with either wortmannin or LY294002 was effective in blunting the anorectic effect of i.c.v. injection of leptin. As evidence that LepR signaling recruits PI3K in hypothalamic neurons, these authors also showed an increase in PI3K activity associated with IRS-2 at 30 min after i.p. injection of leptin (28). These results revealed that PI3K in the brain is required for the short-term effects of leptin on food intake regulation.

Additional studies have confirmed and extended these findings. Of note, it was shown that leptin-induced activation of PI3K in the hypothalamus recruits phosphodiesterase 3B (PDE3). PDE3 counterbalances the activity of adenylyl cyclases, which are responsible for the synthesis of the second messenger cAMP, whereas PDE3 induces cAMP clearance (29). Using i.c.v. administration of a PDE3 inhibitor, cilostamide, the reduction in food intake and body weight observed after leptin treatment was prevented by the inhibition of the PI3K-PDE3 pathway.

As previously mentioned, leptin administration inhibits the expression of the orexigenic NPY. Leptin- responsive NPY cells are found in the ARH and coexpress agouti-related peptide (AgRP) (5-7). The involvement of PI3K in this group of cells was investigated in rats subjected to a 52-h fast (30). The fasted rats showed increased NPY and AgRP mRNA levels, compared to *ad libitum* fed animals. As predicted, i.c.v. administration of leptin over 12-h intervals blocked the fasting-induced increase in NPY and AgRP expression. However, pretreatment with LY294002 blunted the effects of leptin in the expression of NPY and AgRP.

PI3K INTEGRATES THE EFFECTS OF LEPTIN AND INSULIN IN THE REGULATION OF FOOD INTAKE

Insulin also acts in the brain to regulate food intake. For example, administration of insulin in rats causes a significant reduction in food intake and body weight (31). This effect was completely prevented by the administration of PI3K inhibitors, wortmannin and LY294002, one hour before insulin treatment (31). Furthermore, insulin treatment induces phosphorylation of IRS-1, IRS-2, and Akt in the hypothalamus and increases the immunoreactivity of PtdIns 3,4,5-triphosphate specifically in the ARH (31). These results suggest that PI3K is a common intracellular signaling pathway required for the hypothalamic effects of either leptin or insulin in food intake regulation.

Although PI3K seems to be required for the anorectic effects of insulin and leptin, this fact does not imply that PI3K is activated in the same way by both hormones in defined populations of hypothalamic neurons.

In this regard, Xu and cols. (25) generated a mouse model, which expresses the enhanced green fluorescent protein (EGFP) fused to the pleckstrin homology (PH) domain from Grp1 [EGFP-PH(Gpr1)]. Following PI3K generation of PtdIns 3,4,5-triphosphate, the EGFP-PH(Gpr1) complex translocates to the cell membrane, allowing the visualization of neurons in which the PI3K signaling pathway is activated (25). Using this reporter mouse, these authors showed that both leptin and insulin activate the PI3K pathway in POMC neurons. But, while insulin induces the activation of PI3K in AgRP neurons, leptin shows an opposite effect. Interestingly, the action of insulin and leptin in POMC neurons, and of insulin in AgRP neurons, was shown to be direct in those cells. However, the inhibitory effect of leptin on AgRP neurons was blocked by inhibitors of synaptic transmission. These results indicate that leptin recruits another population of neurons to affect PI3K activity in AgRP neurons (25).

PI3K SIGNALING MEDIATES LEPTIN'S EFFECTS ON GLUCOSE HOMEOSTASIS

Experimental evidence also indicates that the PI3K signaling pathway in hypothalamic neurons mediates leptin's effects on glucose homeostasis. Using stereotaxic techniques, leptin receptor-deficient Koletsky (fa^k/fa^k) rats received adenoviral gene therapy to induce the expression of LepR in the ARH (32). The virus-induced LepR signaling in mediobasal hypothalamic neurons markedly increased the insulin tolerance of the fa^k/fa^k rats. On the other hand, i.c.v. administration of LY294002 attenuated the improvement in insulin tolerance of rats submitted to the adenoviral therapy, suggesting that the brain-mediated effects of leptin on glucose homeostasis involve induction of the PI3K signaling pathway. Accordingly, virus-induced expression of a constitutively active Akt in the hypothalamus recapitulated the insulin-sensitizing effects of adenoviral-induced LepR gene expression (32). However, it is worth mentioning that this approach may have induced LepR expression in cells that normally do not express this receptor. Additionally, the degree of gene expression achieved is probably not similar to the physiological levels observed in wild-type rats. Therefore, although these results highlight a potential role of leptin-induced PI3K signaling in the hypothalamus to control glucose homeostasis, additional studies using alternative methods are necessary to establish the physiologic relevance of PI3K signaling pathways in leptin-induced regulation of insulin sensitivity.

More recently, it was demonstrated that the effects of i.c.v. injection of leptin on improving insulin sensitivity in skeletal muscle is also dependent on PI3K signaling (33). Administration of LY294002 blocked leptin's effect on improving glucose tolerance and on the phosphorylation of AMP-activated protein kinase (AMPK)/acetyl-CoA carboxylase pathway in the soleus muscle of rats. Therefore, the leptin-induced activation of the PI3K signaling pathway in the hypothalamus seems to modulate energy sensing pathways, e.g., AMPK signaling, in peripheral tissues, and ultimately control glucose homeostasis.

THE PI3K SUBUNITS

One challenge in designing studies to investigate the physiologic relevance of PI3K signaling using genetically modified mouse models is that the PI3K complex is composed of several subunits encoded by different genes. So far, only a subset of the PI3K subunits has been targets of genetic manipulation to investigate the role played by PI3K signaling in the regulation of energy balance. Three classes of PI3K have been described: Class I, Class II and Class III. Each class shows different molecular structures and lipid substrate preferences. Class I PI3Ks have great affinity to phosphorylate PtdIns 4,5-diphosphate into PtdIns 3,4,5-triphosphate. Class II PI3Ks phosphorylate PtdIns 4-phosphate into PtdIns 3,4-diphosphate. Finally, Class III PI3Ks phosphorylate PtdIns into PtdIns 3-phosphate. The downstream targets change according to the domain activated by different 3-phosphoisositides. For example, PtdIns 3,4,5-triphosphate and PtdIns 3,4-diphosphate recognize, bind, and regulate proteins that contain the PH domain (Figure 1), whereas proteins that contain the phox homology (PX) domain and the FYVE domain are regulated by PtdIns 3-phosphate (34).

Very little is known about the involvement of Class II and Class III PI3Ks in the regulation of leptin's effects. On the other hand, several studies have investigated the possible link between leptin and Class I PI-3Ks (Table 1). Class I PI3Ks are further sub-classified into Class IA and Class IB PI3Ks. The Class IA PI3Ks are enzymatic complexes composed of catalytic and regulatory subunits. The catalytic subunits are subdivided into p110α (encoded by the gene *Pik3ca*), p110β (encoded by the gene *Pik3cb*), and p110δ (encoded by

the gene *Pik3cd*). The regulatory subunits consist of p85α, p55α, p50α (all three encoded by the gene *Pik3r1*), p85β (encoded by the gene *Pik3r2*), and p55γ (encoded by the gene *Pik3r3*). The Class IB PI3Ks are composed of the p110γ catalytic subunit (encoded by the gene *Pik3cg*) and the p101 regulatory subunit (encoded by the gene *Pik3r5*). Class IA PI3Ks are recruited by receptors that display tyrosine kinase activity and by Ras, whereas the Class IB PI3Ks are downstream of G protein-coupled receptors (GPCRs) and Ras (34). The insulin receptor has intrinsic tyrosine kinase activity and, as mentioned, LepR signaling recruits JAK2. Therefore, it is likely that both hormones recruit primarily the Class IA PI3Ks. Nonetheless, it is not possible to rule out an involvement of leptin and insulin with other PI3K classes. The leptin- and insulin-induced activation of Class IA PI3Ks involves the phosphorylation of IRS-1 or IRS-2, which bind to the regulatory PI3K subunits and induce the activation of the PI3K complex (Figure 1).

The disruption of either catalytic or regulatory subunits can interfere with PI3K activity (Table 1). However, the regulation of PI3K is very complex, and the consequences of manipulations in specific subunits may generate unexpected phenotypes. One good example is the mouse models carrying genetic deletions of the p85α or p85β regulatory subunits. Despite the fact that PI3K is a signaling pathway important for insulin to regulate glucose homeostasis, the genetic disruption of genes that encode the p85 regulatory subunits increases insulin sensitivity and causes hypoglycemia and hypoinsulinemia (34-39).

To investigate the role of PI3K signaling in POMC neurons, Hill and cols. (26,40) generated a conditional knockout mouse in which the gene encoding the p85α, p55α and p50α regulatory subunits was selectively disrupted in POMC cells. These authors showed that despite the fact that POMC-specific *Pik3r1* deletion did not change the leptin-induced depolarization and increased firing rate in these cells (26), the female mice were resistant to a diet-induced obesity (40). Nevertheless, no protection against obesity induced by a high-fat diet was observed in male mice carrying the same mutation (40).

Hill and cols. (26) also crossed the POMC-specific *Pik3r1* knockout mice with animals that carry a global deletion in the gene that encodes the p85β regulatory subunit, generating mice lacking p85α and p85β Class IA PI3K regulatory subunits in POMC cells. These authors observed that, in intact POMC neurons, leptin induces a depolarization that can be verified by increases in firing rate and inward current, as well as by a decrease in the whole-cell input resistance. However, in mice lacking the PI3K regulatory subunits in POMC cells, leptin failed to change the membrane potential and to induce inward current in those neurons (Table 2). Furthermore, the deletion of *Pik3r1* and *Pik3r2* genes in POMC cells prevented the reduction in food intake and body weight caused by acute i.c.v. injection of leptin (26). These data suggest that leptin directly excites POMC neurons via a PI3K-dependent mechanism and that deletion of both *Pik3r1* and *Pik3r2* is required to completely disrupt PI3K activity in POMC neurons. Nonetheless, no changes were observed in the long-term regulation of body weight and metabolism (26). These results also suggest that PI3K is important to mediate the acute effects of leptin, but other signaling pathways, possibly JAK2/STAT3, are necessary for the long-term regulation of energy homeostasis. It is important to mention that the use of animals carrying mutations in PI3K regulatory subunits in studies aiming to evaluate the role of PI3K in energy homeostasis may produce ambiguous results (Table 1). As demonstrated before, mouse mutants of p85α or p85β subunits have a reduced body size and increased insulin sensitivity (34,35,39).

THE CATALYTIC SUBUNITS OF CLASS IA PI3Ks

A variety of genetic mouse models with deletions in the catalytic subunits of Class IA PI3Ks has been produced (Table 1). Mice carrying a disruption in the gene that encodes the p110δ catalytic subunit are viable and show no changes in the regulation of energy balance, but they present several immunological problems and abnormalities in B cells, T cells, and neutrophils (41). This phenotype is in accordance with the sites of p110δ expression in the body. While p110α and p110β are ubiquitously distributed among all tissues, the p110δ mRNA is expressed predominantly in leukocytes (42). Thus, considering the brain as the primary target of leptin in energy homeostasis, the role played by the p110δ PI3K catalytic subunit in leptin signaling is likely negligible.

The full-body knockout of p110α or p110β catalytic subunits causes embryonic lethality (34,43,44). In order to study the physiological role played by the p110α PI3K subunit, Foukas and cols. (45) generated a genetically modified mouse that display reduced p110α acti-

vity ($p110\alpha^{ko}$ mice). These mice showed reduced body weight and body length, glucose and insulin intolerance, higher levels of insulin and leptin, and an increased food intake and body fat mass (45). Using different approaches, other groups also showed that the p110α PI3K catalytic subunit is essential for proper growth factor signaling (46). Thus, the p110α PI3K catalytic subunit seems to be critical for metabolism regulation and growth (Table 1).

Recently, two studies specifically focused on the physiological role played by PI3K catalytic subunits in specific populations of hypothalamic neurons (40,47). In these studies, the p110α subunit was selectively deleted from POMC cells or from cells expressing the steroidogenic factor 1 (SF-1). In the brain, the expression of SF-1 is restricted to neurons located in the VMH, including those that express LepR (47). Deletion of p110α in POMC neurons, but not in SF-1 cells, increased the body weight in mice consuming a regular chow diet. However, the deletion of p110α in either POMC or SF-1 cells increased the susceptibility to diet-induced obesity. The food intake remained unchanged, whereas decreased energy expenditure was observed in both conditional knockout models. Also, the p110α deletion in POMC neurons reduced insulin sensitivity without affecting the glucose tolerance of these mice (40). Similarly to the results observed after deletion of p85α and p85β in POMC cells, the disruption of p110α in the VMH decreased the acute metabolic responses observed after i.c.v. leptin administration (47). Overall, these findings indicate that the activity of p110α in leptin responsive neurons is required for the regulation of energy balance and glucose homeostasis (Table 1). Furthermore, these studies demonstrated that body weight changes in mice carrying a conditional deletion of p110α were primarily caused by changes in energy expenditure, instead of food intake. In particular, Xu and cols. (47) showed that the lack of p110α in SF-1 cells blunted the acute increase in energy expenditure, usually observed after exposure to a high-fat diet. Thus, PI3K signaling in the hypothalamus potentially modifies the energy balance through the regulation of either food intake and/or energy expenditure.

Although the aforementioned information strongly indicates that the p110α catalytic subunit is essential for the regulation of energy metabolism, there is still a debate about the role played by other PI3K catalytic subunits. For example, using pharmacological inhibitors specific for each PI3K catalytic subunit, Knight and cols. (2) showed that only the p110α subunit is required for the insulin-induced PI3K activation in cultured cells, whereas the other catalytic subunits are dispensable for the same effect. Nonetheless, p110β and p110δ maintained a basal level of PtdIns 3,4,5-triphosphate, setting a phenotypic threshold for p110α activity in myotubes, but not in adipocytes in cell culture (2). Also, experiments *in vivo* using specific pharmacological inhibitors showed that inhibition of p110α, but not p110β, blocks the acute effects of insulin (2). However, another report found that although p110α is required for insulin-stimulated phosphorylation of Akt in several cultured cell lines, the p110β and p110δ subunits are also necessary for insulin signaling in HepG2 hepatoma cells (48). In accordance, Jia and cols. (49) produced a conditional knockout mouse, in which the p110β subunit was ablated specifically from the liver. These mice displayed impaired insulin sensitivity and glucose homeostasis, but no changes in insulin-induced phosphorylation of Akt in the liver (49). In another study, the role played by the p110β subunit was studied in mutant mice that express a catalytically inactive *Pik3cb* gene (50). These mice survived to adulthood, but they displayed insulin resistance and reduced body growth, suggesting that the p110β PI3K Class IA subunit is also involved in growth and metabolism (50).

As mentioned before, the hypothalamus is the main region for leptin to regulate energy homeostasis (12). However, most of the studies that assessed the role of PI3K catalytic subunits using pharmacological inhibitors evaluated other tissues. To understand the importance of individual PI3K catalytic subunits in the hypothalamus, Tups and cols. (51) immunoprecipitated hypothalamic samples using an antibody against the p85 subunit. These authors observed that PI3K activity was reduced by 65% using a p110α pharmacological inhibitor or by 35% using a p110β selective inhibitor. In the same study, insulin was administered i.c.v. in rats. It was observed that insulin-induced phosphorylation of Akt in the hypothalamus can be partially blocked by inhibitors of either p110α or p110β, whereas the administration of both inhibitors completely prevented the insulin signaling (51). Besides, the combined i.c.v. administration of p110α and p110β inhibitors blocked the acute anorexigenic action of a combination of leptin and insulin (51). Therefore, this study suggests that the inhibition of both p110α and p110β subunits is required for completely blocking the effects of i.c.v. administration of insulin or leptin.

In a recent study, the authors generated a mouse model carrying a conditional deletion of p110α or p110β specifically from cells that express POMC or AgRP (52). As opposed to what was previously suggested, these authors showed that the p110β subunit exerts a dominant role over p110α in the regulation of energy homeostasis. They showed that p110β inactivation in POMC neurons induces an increase in food intake and fat mass in mice exposed to a regular chow diet and to a high fat diet. In contrast, the same study showed that p110α deletion from POMC cells increased the fat mass only in mice consuming a high fat diet. In AgRP neurons, the deletion of p110β, but not p110α, induced a decrease in body weight, fat mass and food intake. This study also emphasized the importance of the p110β subunit for the electrophysiological properties of these neurons, although some controversial points were presented. For instance, POMC-specific deletion of p110α (POMCp110α *null*) created a more hyperpolarized resting membrane potential, compared to intact POMC neurons (52). Apparently, these changes did not interfere with leptin activity, since leptin depolarized POMCp110α *null* neurons and increased their spike firing frequency, as observed in intact POMC neurons. However, while 30% of intact POMC cells were responsive to leptin, 53% of POMC p110α *null* neurons depolarized in response to leptin administration. Therefore, whether deletion of p110α subunit from POMC neurons causes any effect on leptin's action is still controversial. Besides, the deletion of the p110β subunit from POMC neurons (POMC p110β *null*) blocked the depolarizing effects of leptin in these cells. Intriguingly, 40% of the POMC p110β *null* neurons were hyperpolarized by leptin (resting membrane potential -50 ± 1 mV, responsive cell hyperpolarized about -6 ± 1.7 mV). Additionally, insulin hyperpolarizes a subset of POMC neurons, an effect that is blocked by selective deletion of either p110 subunit from these neurons. This indicates that both p110α and p110β subunits may contribute to the electrophysiological properties of insulin in POMC neurons (52).

Another controversial issue presented by Al-Qassab and cols. (52) is that leptin did not evoke any response in AgRP neurons, including cells from control or from conditional knockout mice, even though the literature clearly shows the involvement of leptin on NPY/AgRP neurons (5,6,25). The small number of neurons assessed (n = 7) might explain the lack of response in these cells. In contrast, the authors demonstrated that insulin depolarized approximately 30% of the AgRP neurons, while insulin hyperpolarized both AgRPp110α *null* and AgRPp110β *null* neurons (52). Hence, although this study showed that the deletion of p110α caused fewer changes in the regulation of long-term energy homeostasis compared to p110β, both subunits seem to play a role in the leptin- and insulin-evoked electrophysiological responses in POMC and AgRP neurons.

TARGETING PTEN: ANOTHER WAY TO STUDY THE PI3K PATHWAY

As mentioned before, while PI3Ks phosphorylate PtdIns at the 3-hydroxyl group of the inositol ring, PTEN is responsible for its dephosphorylation. Thus, changes in the activity of PTEN are expected to dramatically affect PI3K signaling. Following this idea, Plum and cols. (53) generated a conditional knockout mouse lacking the *Pten* gene in POMC cells (PPKO mice). These mice exhibited an enhancement of PtdIns signaling in POMC cells. The PPKO mice exhibited hyperphagia and developed a sexually dimorphic diet-sensitive obese phenotype, accompanied by the resistance to leptin's activity *in vivo*, despite leptin-stimulated STAT3 phosphorylation in POMC neurons being unaltered (Table 1). PPKO mice exhibited a marked hyperpolarization and reduced basal firing rate in POMC neurons resulting from increased KATP activity. Leptin induced a small depolarization in PPKO POMC neurons from -58 ± 3 mV to -54 ± 2 mV, but this effect was not sufficient to elicit electrical activity (Table 2). However, the reversal of the increased KATP current in PPKO neurons by tolbutamide restored the ability of leptin to increase firing in these cells. In addition, LY294002 normalized both the amplitude of KATP conductance and the firing rate of the PPKO POMC neurons. Importantly, blocking KATP channels by i.c.v. administration of tolbutamide reversed the hyperphagic phenotype observed in PPKO mice. These findings suggest that PI3K-dependent regulation of KATP channels in POMC neurons plays a central role in the regulation of energy homeostasis (53).

In another study from the same group, PTEN was disrupted in LepR expressing cells ($Pten^{\Delta ObRb}$ mice). $Pten^{\Delta ObRb}$ mice showed enhanced PI3K activity, a higher glucose and insulin tolerance, and a reduced

adiposity as a result of increased energy expenditure (54). Part of the changes in energy expenditure in the $Pten^{\Delta ObRb}$ mice was caused by white adipose tissue (WAT) transdifferentiation. The perigonadal WAT of $Pten^{\Delta ObRb}$ mice exhibited markers of brown adipose tissue, e.g., increased mitochondrial content and uncoupling protein-1 expression. Interestingly, leptin was required for all these effects because the conditional disruption of PTEN in leptin-deficient mice did not affect their metabolism or WAT morphophysiology (54).

Although the manipulation of PTEN offers another way to study the PI3K pathway, through the hyperactivation of PtdIns signaling, it is important to mention that PTEN possesses physiological properties beyond those related to its lipid phosphatase activity (55). In addition, PTEN can affect other signaling pathways, including the mitogen-activated protein (MAP) kinase pathway (56). Therefore, the results from studies manipulating PTEN to investigate the PI3K pathway must be analyzed carefully because of the possible nonspecific changes caused by disruption of PTEN.

Table 1. List of studies that investigated the physiological role played by different Class IA PI3K subunits and by PTEN in the regulation of energy homeostasis

Targeted PI3K subunit	Metabolic phenotype	Acute sensitivity to leptin	References
Global deletion			
p85α + p55α + p50α (all encoded by *Pik3r1* gene)	perinatal lethality; hypoglycemia; liver necrosis	N.D.	(37)
p85α	↑ insulin sensitivity; hypoglycemia; ↑ glucose transport	N.D.	(39)
p55α + p50α	↑ insulin sensitivity; ↑ glucose transport; ↓ body fat and leptin; ↔ food intake	N.D.	(36)
p85β	Hypoinsulinemia; hypoglycemia; ↑ ınsulin sensitivity; ↓ body size	N.D.	(35)
p110α	Embryonic lethality (between E9.5 and E10.5)	N.D.	(44)
p110β	Embryonic lethality (most until E3.5)	N.D.	(43)
p110δ	No metabolic abnormality reported	N.D.	(41)
Heterozygous mutants or mice carrying reduced activity			
p85α + p55α + p50α	↑ insulin sensitivity; ↑ glucose tolerance; ↓ glycemia	N.D.	(38)
p110α	↓ somatic growth; hyperinsulinemia; ↓ glucose tolerance; ↓ body weight; hyperphagia; ↑ adiposity; ↑ leptin levels	N.D.	(45)
p110β	↓ somatic growth; ↓ insulin sensitivity; ↓ glucose tolerance; ↔ food intake	N.D.	(50)
Cell-specific deletions			
p85α + p55α + p50α in POMC cells; global p85β	↔ body weight and adiposity; ↔ food intake; ↔ energy expenditure	Blunted leptin-induced changes in food intake and body weight	(26)
p85α + p55α + p50α in POMC cells	Resistance to a diet-induced obesity in females; ↔ body weight and adiposity in males; ↓ insulin levels; ↔ glycemia; ↔ glucose and insulin tolerance	N.D.	(40)
p110α in POMC cells	↑ body weight and adiposity in males and females (40); ↑ susceptibility to a H.F.D. (52); ↓ energy expenditure (40); ↔ food intake; ↓ insulin sensitivity (40)	No significant effects (52)	(40,52)
p110α in AgRP cells	↔ body weight and adiposity; ↔ food intake; ↔ glucose homeostasis	No significant effects	(52)
p110α in SF-1 cells	↔ body weight in regular diet; ↑ susceptibility to a H.F.D.; ↔ food intake; ↓ energy expenditure; ↓ thermogenic response to H.F.D.; ↔ insulin sensitivity	Blunted leptin-induced changes in food intake and body weight	(47)
p110β in POMC cells	↑ adiposity; ↑ body weight in H.F.D.; ↑ food intake; ↔ energy expenditure; ↔ glucose homeostasis; ↑ body length	Blunted leptin-induced changes in food intake	(52)
p110β in AgRP cells	↓ body weight and adiposity; ↓ food intake; ↔ energy expenditure; ↔ glucose homeostasis	↑ sensitivity to anorectic effects of leptin	(52)
p110β in the liver	↓ insulin sensitivity; ↓ glucose tolerance; hyperinsulinemia; ↑ leptin levels	N.D.	(49)
PTEN in POMC cells	↑ adiposity; sexually dimorphic susceptibility to a H.F.D.; ↑ food intake; ↔ energy expenditure; ↑ body length	Blunted leptin-induced changes in food intake	(53)
PTEN in LepR cells	↓ body weight and adiposity; ↑ energy expenditure; ↔ food intake; ↑ insulin sensitivity; ↑ glucose tolerance; ↑ glucose uptake	↑ leptin-induced sympathetic nervous activity in fat depots	(54)

N.D.: not determined.

Table 2. Electrophysiological properties of intact neurons and of neurons with specific Class IA PI3K subunit deletions

	RMP	Response to leptin	Response to insulin	References
Intact neurons				
POMC neurons	-40 to -45 mV	Depolarization (+10 to +20 mV)	N.D.	(27)
POMC neurons	-44.8 ± 0.8 mV	Depolarization (80%, +6.2 ± 0.5 mV)	Hyperpolarization (63%, -7.7 ± 1.2 mV)	(26)
POMC neurons	-50 ± 1 mV	Depolarization (30%, +4.5 ± 1.3)	Hyperpolarization (63%, -7.1 ± 1.1)	(52)
AGRP neurons	-50 ± 1 mV	No response	Depolarization (30%, +3.8 ± 0.9)	(52)
Cell-specific deletions				
p85α + p55α + p50α (encoded by *Pik3r1* gene) in POMC cells	Similar to intact neurons	Similar response as observed in POMC intact neurons	N.D.	(26)
p85α + p55α + p50α in POMC cells plus p85β all tissues	Similar to intact neurons	(83%, no response)	(90%, no response)	(26)
p110α in POMC cells	-56 ± 1 mV	Depolarization (53%, +6.3 ± 1.9)	No response	(52)
p110β in POMC cells	-50 ± 1 mV	Hyperpolarization (37%, -6.1 ± 1.7)	No response	(52)
PTEN in POMC cells	-58 ± 3 mV	Depolarization (66%, +4 ± 2)	N.D.	(53)
p110α in AgRP cells	-48 ± 1 mV	No response	Hyperpolarization (100%, -4.0 ± 0.6)	(52)
p110β in AgRP cells	-50 ± 1 mV	No response	Hyperpolarization (54%, -5.2 ± 1.4)	(52)

The percentage represents the proportion of neurons that responded to a specific drug application. RMP, resting membrane potential. N.D., not determined.

PI3K: A POTENTIAL LINK BETWEEN METABOLISM AND CANCER

Obesity, which is a condition of hyperleptinemia (7), has a strong correlation with the onset of certain cancers. According to a longitudinal study that evaluated more than 900,000 American adults, overweight or obesity accounted for 14% and 20% of all cancer deaths in men and women, respectively, after a follow-up period of 16 years (57). Interestingly, a high association between the development of various types of cancer and abnormalities in the PI3K pathway has also been reported (45,46,49,58). For instance, somatic missense mutations in the *Pik3ca* gene occur at a high frequency in many types of human cancers (3). The relationship between PI3K and the development of cancers indicates that drugs that affect the activity of PI3K can be a promising anti-cancer therapy (1-3). Thus, it is possible that the PI3K signaling pathway is an important link between obesity, leptin and increased risk of cancer (59). Nonetheless, other intracellular pathways, including JAK/STAT3 and MAP kinase, may also be involved in leptin-induced development of certain types of cancer (60).

CONCLUSIONS AND FUTURE PERSPECTIVES

In the present review, we discussed the evidence offered by recent findings that the PI3K signaling pathway is required for leptin-induced regulation of energy homeostasis. Manipulation of the PI3K signaling pathway in specific populations of leptin-sensitive neurons causes different biological effects, possibly related to the role played by particular neurons in leptin physiology. Although the role played by PI3K signaling in each defined population of leptin-sensitive neurons is of fundamental importance, potential drugs that target PI3K subunits are likely to produce a systemic effect. Therefore, broader strategies to study leptin and PI3K will expand our knowledge on the whole-body effects of PI3K in the regulation of energy homeostasis. Moreover, the various subunits of PI3K appear to differentially regulate energy homeostasis, but their exact role and their physiological relevance still need to be further assessed in more detail. Studies that directly assess the possible involvement of Class IB, Class II and Class III PI3Ks in leptin's physiology will be informative. Finally, the PI3K pathway seems to be a very good candidate for regulating the rapid (acute) effects of leptin, whereas the JAK2/STAT3 pathway plays a dominant role in the long-term regulation of energy homeostasis. However, changing the acute cellular response to leptin may create a chronic condition that will ultimately affect the long-term regulation of energy homeostasis. Understanding how these conditions develop and how PI3K interacts with other intracellular pathways represents important challenges for future studies.

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