Insulin and its evolving partnership with leptin in the hypothalamic control of energy homeostasis

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Despite an alarming increase in the burden of obesity worldwide, body adiposity seems to be a regulated physiological variable. Regulation of adiposity occurs through a classical endocrine feedback loop, in which the pancreatic β-cell-derived hormone insulin and the adipocyte-derived hormone leptin signal the status of body energy stores to the hypothalamus. Recent advances in our understanding of the signal transduction mechanisms used by insulin and leptin in the hypothalamus to modulate neuronal firing suggest that intracellular cross-talk occurs at several levels and is a potentially important determinant of regulated body weight. These pathways are thus an attractive target for pharmacological intervention in the treatment of obesity.

Insulin as a central effector of energy homeostasis

Although the cloning of leptin [5] and the elucidation of its crucial role in energy homeostasis [6] were seminal events in energy homeostasis research, Woods, Porte and their coworkers [7–9] demonstrated the existence of neural circuits that respond to insulin and regulate food intake and adipose mass many years before leptin was discovered. Work conducted in the late 1970s and early 1980s revealed that chronic infusion of insulin into the cerebral ventricles of baboons reduces food intake and body adiposity [7], providing crucial early evidence in support of the feedback loop model of energy homeostasis on which current models are based [4]. Indeed, insulin shares characteristics with leptin that are requisite for an adiposity signal: it is secreted in proportion to body fat mass [10] and interacts with key neurons found in the hypothalamic arcuate nucleus (ARC) that expresses both insulin [11,12] and leptin [13] receptors (Figure 1).

Although leptin is recognized as a principal regulator of fat mass, the concept that insulin functions as a catabolic ‘adiposity negative feedback signal’ through the regulation of key hypothalamic neurons is relatively less appreciated, even though the strength of evidence in support of this hypothesis is equally as robust as that in support of the role of leptin. Part of the reason for this might be that the notion of insulin as a centrally acting catabolic hormone runs counter to its well-recognized role as the prototypical anabolic (and glucoregulatory) hormone in peripheral tissues. For example, administration of insulin can lead to hypoglycemia and counter-regulatory increases in food intake and, if repeated, can cause weight gain via this mechanism [14]. In addition, insulin functions in the periphery to promote storage of energy in the forms of carbohydrate, protein and fat. A combination of these factors probably explains the general clinical observation that insulin usage can lead to weight gain in individuals with diabetes [15], rather than the weight loss predicted to occur with the administration of an ‘adiposity signal’.

As we elaborate in this review, the idea that central insulin action is fundamentally catabolic (i.e. reducing...
food intake and body weight), whereas its peripheral actions are anabolic (i.e. increasing energy storage and potentially increasing body weight if an individual has too much insulin), is ultimately consistent with, and illustrative of, the concept of an endocrine feedback loop in the control of energy stores [4]. Like most physiological systems, the peripheral and central actions of insulin are balanced and function to promote euglycemia, optimal body composition and reproductive fitness.

The opposing central and peripheral effects of insulin are evident in the condition known as diabetic hyperphagia, a prominent behavioral manifestation of uncontrolled human type I diabetes or experimental diabetes in animals. When β cells have been destroyed, the absolute lack of insulin results in hyperglycemia and an inability to store energy in peripheral tissues, ultimately causing a marked loss of body weight. The lack of catabolic insulin action in the brain, coupled with the decrease in serum leptin that accompanies weight loss, however, leads to marked increases in food intake that compensates, in part, for energy wasting and prevents a more rapid demise. Stated differently, if the ‘catabolic’ tone of hypothalamic insulin and leptin action were not relieved by the absence of these hormones, weight loss would be greater and survival impaired. In fact, either insulin or leptin given systemically or directly into the third cerebral ventricle of rats with streptozotocin-induced diabetes blunts or completely blocks this compensatory hyperphagia [16,17] and results in a more rapid loss of weight. The fact that either hormone given alone can block diabetic hyperphagia is evidence of overlap in their functions in the CNS.

A brief comment on the access of insulin, as a central effector of energy homeostasis, to the CNS is warranted. The hypothesis that circulating insulin provides a physiological signal to brain areas involved in energy homeostasis implies a mechanism whereby this peptide traverses the blood–brain barrier (BBB) to enter brain interstitial fluid. Early studies demonstrated that, during intravenous infusion, increases in plasma insulin are paralleled by proportionate (albeit much smaller in absolute terms) increases in cerebrospinal fluid insulin levels in experimental animals [18] and humans [19]. Subsequent studies showed that this insulin uptake process involves a saturable transport mechanism [20] and, combined with the findings that insulin receptors are expressed by endothelial cells including those present in brain capillaries [21], and that these receptors are capable of transendothelial insulin transport [22], led to the conclusion that insulin delivery across the BBB involves an active, receptor-mediated mechanism. Specifically, plasma insulin is proposed to bind to its receptor on the luminal endothelial surface and, after being internalized into an endocytic vesicle (still bound to receptor), is ultimately released intact from the abluminal surface into interstitial fluid [21,23].

The observation in dogs that insulin transport into the brain is attenuated by the consumption of a high-fat diet [24] raises the possibility that acquired reductions in brain

Figure 1. Neurons in the arcuate nucleus (ARC) express insulin and leptin receptors and integrate peripheral signals to maintain energy homeostasis. (a) According to current models, the ARC contains anabolic neurons coexpressing neuropeptide Y (NPY) and agouti-related protein (AgRP) and catabolic neurons expressing pro-opiomelanocortin (POMC). Both types of cell respond to insulin and leptin but do so in the opposite manner: NPY/AgRP neurons are inhibited and POMC neurons are activated. It is thought that this coordinate dual regulation of opposing cell types ultimately regulates complex responses, such as changes in feeding and energy expenditure, to maintain body fat stores. In this model, insulin and leptin have similar roles. Reproduced, with permission, from Ref. [70]. (b) Radiolabeled insulin administered to rats binds to neurons found in the ARC (oval outline), as well as various other brain areas. Image is in pseudocolor with highest intensity binding indicated in purple. (c) Radiolabeled leptin administered to rats binds primarily to neurons found in the ARC (oval outline). Image is in pseudocolor with highest intensity binding indicated in purple.
insulin delivery might respond with an increase in body weight. The extent to which a regulated transport mechanism mediates insulin delivery to specific key neuronal targets involved in energy homeostasis is, however, uncertain. This is an especially important issue with respect to the hypothalamic ARC. This brain area is situated adjacent to the median eminence, which lacks a BBB, and available data have yet to determine whether or not ARC neurons are protected by the BBB [25,26]. Indeed, capillary permeability is reported to vary extensively across the ARC itself, being greater in its medio-basal than its lateral aspect [27]. Thus, additional studies are needed to clarify whether circulating insulin gains access to ARC neurons by simple diffusion or via a more complex, regulated transport mechanism.

Current model of energy homeostasis: proposed role of insulin

A current and well-accepted model of the regulation of adipose tissue stores has been reviewed in detail elsewhere (Figure 1a) [3,4]. In this model, both leptin, the adipocyte-derived hormone, and insulin, the pancreatic β-cell-derived hormone, are proposed to function as adiposity signals in the feedback regulation of adipose mass [3,4]. Both hormones circulate in the bloodstream in proportion to body fat mass [10,28] and regulate the activity of neurons found in regions of the brain associated with body weight regulation. Neurons found in the hypothalamic ARC express receptors for, and bind to, both insulin (Figure 1b) and leptin (Figure 1c) [12,13], and are increasingly well-characterized targets of both hormones [29–31]. ARC neurons project to other key brain areas and are thought to function as primary neurons in a series of neural circuits that regulate food intake, energy expenditure, hypothalamus–pituitary function and sympathetic outflow [32].

Situated in the ARC are at least two distinct neuronal cell types (Figure 1a) that respond to adiposity signals and exert opposing effects on energy balance: one is anabolic in nature, whereas the other is catabolic [4,31]. Anabolic neurons are those that, when activated, promote an increase in food intake and a decrease in energy expenditure, leading to the storage of energy. In the ARC, these neurons coexpress the orexigenic neuropeptides neuropeptide Y (NPY) and agouti-related protein (AgRP). NPY/AgRP neurons are inhibited by rising leptin and insulin levels, and this inhibition is thought to mediate some of the anorectic actions of these hormones. For example, insulin infusion directly into the third cerebral ventricle reduces expression of the NPY gene (a marker of neuronal activation) in fasted or STZ diabetic rats, which in effect blocks the increase in NPY gene expression that would otherwise occur in these rats [33]. Conversely, antisense RNA ‘knockdown’ of hypothalamic insulin receptors causes an increase in both food intake and expression of the NPY and AgRP genes [34].

These findings add physiological relevance to the observation that insulin given intracerebroventricularly (i.c.v.) reduces food intake in baboons [7], sheep [35] and rodents [36,37] by suggesting that tonic action of insulin to inhibit the production and release of a potent orexigenic neuropeptide normally constrains food intake. Furthermore, in an isolated neuronal preparation, the firing rate (and thus release of neuropeptide) of a subset of ARC neurons proposed to be NPY/AgRP neurons was found to be reduced by insulin treatment and similar effects were seen after leptin treatment [29,30]. The effect of both insulin and leptin to hyperpolarize this population of hypothalamic neurons is dependent on signaling through phosphatidylinositol 3-kinase (PI3K), a classical insulin signaling target, and on the opening of ATP-sensitive K+ channels [29,30].

Acting in opposition to the NPY/AgRP neuron is the catabolic neuron, which expresses pro-opiomelanocortin (POMC). According to current models, these cells release the anorexigenic neuropeptide α-melanocyte-stimulating hormone when stimulated by insulin and leptin, and thereby promote a decrease in food intake and body weight. Insulin, like leptin, increases POMC gene expression when given i.c.v. [38] and activates downstream melanocortin ‘target’ neurons [38].

Thus, POMC and NPY/AgRP neurons comprise two primary but opposing sets of neurons found in the ARC that are reciprocally regulated by both insulin and leptin, establishing a system of dual anabolic and catabolic regulation (Figure 1a) [4]. These ARC neurons are especially interesting because of their proposed role as the primary neurons that respond directly to insulin and leptin and that sense input related to body fuel stores to regulate, in turn, downstream neurons and circuits [4]. Many other brain areas are crucial targets of ARC neurons: the identification of these areas is key, because it is ultimately the net activity of these neural circuits that integrates various inputs pertinent to energy homeostasis [32]. Notably, both insulin and leptin have been implicated in the cognitive aspects of feeding (reward, learning and memory) via receptors in cortico-limbic sites [39], an emerging area of interest that could be particularly relevant to populations that become obese in association with the consumption of highly palatable diets.

Experimental models supporting a role for insulin

First proposed in the 1970s, the hypothesis that insulin acts in the CNS to control energy homeostasis has enjoyed a resurgence of interest in response to several recent models of insulin action in the CNS. Interestingly, nematodes such as Caenorhabditis elegans express homologs of insulin, insulin receptors and insulin signal transduction molecules [40,41]. Because these organisms regulate fat stores but do not express leptin signaling homologs, these observations suggest that, from an evolutionary perspective, the insulin signaling system might have a more primitive role in energy homeostasis than the leptin pathway.

Null mutants of insulin signal transduction molecules such as DAF-2, an insulin receptor homolog, show increased fat storage in the intestine, increased longevity and reduced reproductive function (Figure 2) [40,41]. This outcome seems to indicate that insulin signaling cascades have a role in the nervous system of C. elegans, where insulin action has the ‘catabolic’ effect of limiting energy storage, because the mutants have larger fat deposits. It is
hypothalamus are similar (Figure 1a) [49]. In brief, ligand physiological responses to insulin and leptin in the transduction pathways [47,48], these cellular and molecular approaches each support a role for insulin in the regulation of hypothalamic neurons and therein of energy homeostasis. Given the strength of this evidence and similarities in the action of insulin and leptin in the hypothalamus, an exploration of the intracellular signal transduction mechanisms used by these two hormones in this brain region is warranted. Given similarities in the action of insulin and leptin in this brain region is warranted.

Also noteworthy that these primitive animals do not seem to regulate carbohydrate flux actively, suggesting that insulin had an important role in the control of energy balance, life cycle and reproduction before the evolution of a sophisticated insulin-regulated system of glucose homeostasis [40–42]. Likewise, the fruitfly, Drosophila melanogaster, expresses insulin signaling homologs that might have a similar role in the regulation of energy metabolism and lifespan [43].

Although nematodes and flies provide an intriguing evolutionary perspective, recently developed molecular genetics models in rodents have shed additional light on the role played by insulin in both reproduction and energy balance. Both brain-specific knockouts of the insulin receptor and animals lacking insulin receptor substrate-2 (IRS2), a key molecule linking activated insulin receptors to signal transduction via PI3K (Figure 3), show a phenotype of obesity and reproductive dysfunction [44,45]. Similarly, knockdown of insulin receptor expression locally in the mediobasal hypothalamus by RNA antisense technology [34] was found to result in a cumulative food intake of 152% and fat mass of 186% relative to control animals treated with nonsense RNA [34]. The consensus from various molecular genetics approaches is, therefore, that insulin action in the hypothalamus is essential in the regulation of energy homeostasis.

A broader perspective reveals that neurochemical [33,38], electrophysiological [30], behavioral [7,36,37,46] and molecular genetics [34,44,45] approaches each support a role for insulin in the regulation of hypothalamic neurons and therein of energy homeostasis. Given the strength of this evidence and similarities in the action of insulin and leptin in the hypothalamus, an exploration of the intracellular signal transduction mechanisms used by these two hormones in this brain region is warranted.

**Insulin and leptin signal transduction**

Although originally associated with divergent signal transduction pathways [47,48], the cellular and physiological responses to insulin and leptin in the hypothalamus are similar (Figure 1a) [49]. In brief, ligand binding to the heterotetrameric insulin receptor activates an intrinsic tyrosine kinase, leading to the autophosphorylation of several tyrosine residues on the intracellular portion of the receptor (Figure 3). Subsequently, a member of a family of proteins known as IRS molecules is activated by phosphorylation mediated by the insulin receptor. After this tyrosine phosphorylation step, IRS proteins activate the kinase PI3K, which consists of a regulatory subunit (typically called p85) and a catalytic domain (p110). By binding to the p85 subunit, phosphorylated IRS molecules activate the p110 subunit of PI3K, which in turn catalyzes the phosphorylation of phosphatidylinositol (4,5)-bisphosphate to phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P3]. The latter functions as an active signal transduction factor, leading to the activation of several downstream molecules such as 3-phosphoinositide-dependent kinase-1, glycogen synthase kinase-3 and protein kinase B [47,50,51].

Unlike insulin, leptin is a cytokine-like molecule whose receptor is a member of the type 1 cytokine receptor family that includes receptors for ciliary neurotrophic factor, leukemia inhibitory factor and interleukin-6 [48]. Although several splice variants exist, the fully active signaling isoform of the leptin receptor is the long form or ‘B isoform’. Binding of leptin leads to the recruitment of Janus-activated kinase-2 (JAK2), a cytosolic tyrosine kinase (Figure 3). JAK2 functions as an extrinsic tyrosine kinase and phosphorylates tyrosine residues on the intracellular portion of the leptin receptor. Signal transducer and activator of transcription-3 (STAT3) molecules are transcription factors that are activated, in turn, by binding to the activated leptin receptor, whereupon they are phosphorylated by JAK2. Activated STAT3 then dimerizes and translocates to the nucleus to regulate gene transcription. Both the leptin and the insulin receptor also couple to additional signal transduction pathways (including extracellular signal-regulated kinase and mitogen-activated protein kinase pathways) [48,50].

**Cross-talk between leptin and insulin in the hypothalamus**

Much of our knowledge about insulin and leptin signaling arises primarily from studies in peripheral tissues and cell culture. In several of these signaling models, leptin has the ability to activate both the JAK/STAT pathway and the classically insulin-like PI3K pathway [52–55], in a phenomenon referred to as ‘cross-talk’. Given similarities in the hypothalamic responses to insulin and leptin in energy homeostasis, an attractive hypothesis is that this cross-talk also exists in key brain areas such as the ARC [49]. Addressing this possibility, Spanswick, Ashford and their coworkers [29,30] carried out a series of electrophysiological studies in hypothalamic slice preparations, which showed that both leptin and insulin acutely regulate the membrane potential and firing rates of a specific subset of neurons. For both hormones, this effect was dependent on signaling through PI3K [29,30] – a clear demonstration of ‘cross-talk’ in the CNS.

Other studies have addressed the role of hypothalamic PI3K signaling in the regulation of food intake. Our...
research group and others have demonstrated in vivo that insulin activates the IRS/PI3K signaling cascade in mediobasal hypothalamus and, furthermore, that PI3K signaling is required for the inhibition of food intake by insulin administered i.c.v. [37,56]. Studies using an immunohistochemical method to detect the PI3K-catalyzed reaction product PtdIns(3,4,5)P_3 have shown that PI3K seems to be activated in ARC neurons that coexpress IRS2 [57], as predicted [37].

We have also tested the hypothesis that at least some of the hypothalamic effects of leptin might be mediated by PI3K signaling. Like insulin, leptin activates PI3K in hypothalamic neurons and this activation is required for the ability of leptin administered i.c.v. to reduce food intake [58], an observation that has been confirmed by other groups [59]. These findings support a model in which activation of IRS/PI3K signaling in hypothalamic neurons is a crucial event in the ability of adiposity-related hormones to regulate energy homeostasis. Notably, as discussed above, insulin-related signaling in C. elegans and Drosophila also uses a pathway involving IRS and PI3K homologs [40,41].

**Important unanswered questions**

Several important questions regarding hypothalamic insulin/PI3K signaling remain unanswered (Box 1), of which we mention only a few here. First, what are the relative contributions of the PI3K pathway and JAK/STAT signaling to insulin and leptin action and ultimately to integrated energy homeostasis? For example, leptin (ob/ob) or leptin receptor (db/db) mutant mice have a more severe obesity phenotype than do the insulin signaling pathway mutants described above, suggesting that insulin and leptin signaling have differential participation in the processes underlying energy homeostasis. An illustrative example is provided by a germline altered mutant leptin receptor mouse that is genetically defective in its ability to activate STAT signaling [60]. The observations that these mice are hyperphagic and obese (more obese than insulin signaling mutants, but not as obese as db/db control mice) suggest that JAK/STAT signaling has an important role in leptin-mediated control of body adiposity. Unlike db/db mice that lack all leptin receptor function, however, these mice do not express reproductive defects and have an attenuated diabetes phenotype [60]. Thus, STAT3 activation is necessary for some, but not all, leptin receptor functions.

As mentioned above, brain-specific knockouts of the insulin receptor and animals lacking IRS2 show a phenotype of milder obesity with reproductive impairment and glucose intolerance [44,45]. These data support a model in which insulin/PI3K signaling and reproduction might be ‘localized’ predominantly to the NPY/AgRP neurons in the ARC, whereas the control of energy expenditure, food intake and fat mass might rely more heavily on signaling via the POMC neuronal subtype [61]. An alternative, but not mutually exclusive, hypothesis states that the insulin and leptin neuronal signaling pathways are intricately related and, for example, that defects in leptin signal transduction lead to insulin resistance, as is observed in the db/db mouse model [49]. In short, the available information has yet to clarify how both insulin and leptin input to a specific neuron is integrated in the control of either reproduction or energy homeostasis. Given the interrelatedness of these systems, a careful dissection of the individual contributions of physiological insulin and leptin action in the hypothalamus will require novel approaches.
Box 1. Outstanding questions

- How is neuronal input from both insulin and leptin ultimately integrated to regulate energy homeostasis?
- Do insulin and leptin regulate the same or distinct populations of hypothalamic neurons via PI3K?
- Does leptin signal via both PI3K and JAK/STAT in the same neuron?
- Does insulin use signaling pathways in addition to the PI3K pathway in hypothalamic neurons?
- How does PI3K activation couple to neuronal firing in POMC neurons and to neuronal inhibition in NPY/AgRP neurons?
- Does insulin signaling in the hypothalamic extra-arcuate nucleus and non-hypothalamic brain areas contribute to energy homeostasis?
- Because PI3K is a ‘target’ of insulin resistance in peripheral tissues, does hypothalamic insulin resistance at the level of PI3K activation contribute to the pathophysiology of obesity?

A second compelling, and related, fundamental issue is whether PI3K signaling is required for the response of NPY/AgRP neurons, POMC neurons, or both sets of neurons to adiposity signals. Because POMC neurons are activated by insulin and leptin, whereas NPY/AgRP neurons are inhibited, it seems counterintuitive to suggest that both responses involve an ‘insulin-like’ PI3K-dependent mechanism. What key difference might allow this regulation to occur in opposite directions in adjacent cell populations?

Finally, to what extent are the overlapping actions of leptin and insulin in the CNS attributable to intracellular cross-talk at the neuronal level? A principal challenge for this work is to develop key methods with which to examine cellular signaling events and neuronal responses in the context of the most complex of tissues containing numerous cell types and almost infinite cell–cell interactions. Additional unanswered research questions are listed in Box 1.

Hypothalamic insulin signaling and obesity

As in most biomedical research, the ultimate goal underlying studies of hypothalamic insulin action is to generate insight into human disease and its treatment. Human obesity seems to be characterized by hypothalamic resistance to adiposity signals, such as insulin and leptin, in the sense that obese individuals have markedly increased serum insulin [10] and leptin [28] levels, reflecting an increase in body adipose mass. Yet, even in the face of this increase in adiposity signals, food intake remains normal or high, analogous to the hyperinsulinemia and normo- or hyperglycemia of type 2 diabetes. Does this phenomenon represent an acquired metabolic defect in hypothalamic signal transduction?

Such a scheme might involve mechanisms analogous to those involved in peripheral insulin resistance in obesity and diabetes. In peripheral tissues, activation of PI3K in response to insulin is a central event in the activation and translocation of glucose transporter molecules to the cell membrane, which in turn facilitate glucose clearance from the bloodstream [47, 51, 62, 63]. Intravenous fat infusion [64–66], high-fat feeding [67, 68] and obesity [69] per se have each been shown to impair the ability of insulin to activate PI3K and thus glucose transport. Because obesity and type 2 diabetes occur in the same metabolic milieu, it is intriguing to propose that a mechanism similar to that causing type 2 diabetes causes insulin and leptin resistance in the hypothalamus, as has been shown for genetic models of obesity [56]. Thus, demonstration that the classically insulin-like signal transduction pathway involving PI3K is relevant to the CNS control of energy homeostasis might yield new therapeutic opportunities for the treatment of obesity.

Conclusions

A compelling body of work, both historical and more recent, using various model systems and experimental approaches strongly supports the idea that insulin, together with leptin, has an important role in the hypothalamic control of energy homeostasis. Further study of the role of IRS/PI3K signaling in the hypothalamus could reveal new targets for the treatment of obesity and will address outstanding fundamental research issues concerning the cellular organization of essential signaling systems in a complex tissue such as the brain. It is likely that evermore sophisticated experimental approaches will be necessary to understand how complex inputs from the periphery are integrated in the CNS to maintain homeostasis, but such information is needed to improve our understanding of the pathogenesis of common obesity.

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References

10 Bagdade, J.D. et al. (1967) The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. J. Clin. Invest. 46, 1549–1557
48 Banks, A.S. et al. (2000) Activation of downstream signals by the long form of the leptin receptor. J. Biol. Chem. 275, 14563–14572
53 Harvey, J. et al. (2000) Essential role of phosphoinositide 3-kinase in leptin-induced K_{ATP} channel activation in the rat CRI-G1 insulinoma cell line. J. Biol. Chem. 275, 4660–4669
66 Griffin, M.E. et al. (1999) Free fatty acid-induced insulin resistance is associated with activation of protein kinase C and alterations in the insulin signaling cascade. Diabetes 48, 1270–1274
68 Buettner, R. et al. (2000) Correction of diet-induced
