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## Leptin and the CNS Control of Glucose Metabolism

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### Abstract

The regulation of body fat stores and blood glucose levels is critical for survival. This review highlights growing evidence that leptin action in the central nervous system (CNS) plays a key role in both processes. Investigation into underlying mechanisms has begun to clarify the physiological role of leptin in the control of glucose metabolism and raises interesting new possibilities for the treatment of diabetes and related disorders.

### I. INTRODUCTION

Obesity, insulin resistance and type 2 diabetes are growing health concerns and the incidence and prevalence of these diseases is increasing world-wide (175). In the US alone, more than two-thirds of adults are classified as either overweight or obese while more than 23 million people have been diagnosed with type 2 diabetes (117). These numbers are expected to increase in the foreseeable future with costs for obesity in the US alone well in excess of \$100 billion per year (156, 175). Obesity is associated with increased morbidity, because it is a major risk factor for diabetes, cardiovascular disease, and cancer. Diabetes, in turn greatly increases the risk of heart disease and both macro- and micro-vascular disease (175, 202). Given these statistics, the need for a better understanding of how body weight and glucose metabolism are regulated is compelling, as such information is essential if we are to develop new strategies for the safe and effective treatments for obesity and type 2 diabetes.

In studies dating back to the 19<sup>th</sup> century, the French physiologist Claude Bernard observed that puncturing the floor of the fourth ventricle (“pique diabetique”) induced diabetes (18), suggesting that the brain plays an important role in the control of blood glucose. Following the discovery of insulin in the early twentieth century (8), however, research into the regulation of glucose metabolism focused primarily on insulin secretion and on its action in peripheral tissues such as liver, muscle and adipose tissue. One result of this research effort is clear evidence, accumulated over the last forty years, that obesity is associated with insulin resistance in these tissues and that this insulin resistance is a cardinal feature of type 2 diabetes (125). Consequently, pharmaceutical strategies for the treatment of type 2 diabetes have tended to focus on either increasing insulin secretion or improving insulin sensitivity. Yet while a large literature provides insight into the pathogenesis of obesity-associated insulin resistance, a parallel body of evidence suggests that the CNS plays an important role in the regulation of glucose metabolism, and that dysfunction of this control system may contribute to metabolic consequences of obesity (161).

## A. Model of CNS Control of Energy and Glucose Homeostasis

The brain continuously transduces input from neural, hormonal and nutrient-related signals into responses that maintain both energy- and glucose-homeostasis. Afferent signals such as the hormones insulin and leptin convey information to the brain regarding long-term energy stores, while information regarding short-term energy availability is conveyed by nutrient-related signals such as glucose and free-fatty acids (FFA). In response to this input, the brain makes adjustments to output systems that control food intake, energy expenditure, hepatic insulin sensitivity and glucose uptake (Figure 1). To restore homeostasis in the face of reduced energy stores or energy availability for example, the brain initiates responses that promote both positive energy balance (i.e. increased food intake and reduced energy expenditure) and increased nutrient availability (increased endogenous glucose production). Conversely, in times of nutrient abundance and excess energy storage, the brain initiates responses that both promote negative energy balance and limit endogenous glucose production. In view of anatomical overlap in brain areas involved in energy homeostasis and glucose metabolism, dysfunction along a single neuroregulatory pathway can potentially predispose to both obesity and type 2 diabetes; remedying such a defect could therefore be of benefit in the treatment of both disorders. The goal of this review is to highlight recent advances in our understanding of the CNS mechanisms governing glucose metabolism, with a special focus on the adipocyte hormone, leptin.

A large and compelling body of evidence supports the hypothesis that hormonal signals generated in proportion to body fat stores act in the CNS via a negative feedback loop to promote energy homeostasis. Early support for this model was based on the adaptive response to conditions characterized by negative energy balance and weight loss. These include the increase of food intake and reduction of energy expenditure induced by energy deprivation or chronic energy restriction that enables the efficient recovery of lost weight, comprising an important protective mechanism for survival (164). In 1953, Kennedy first hypothesized that body adiposity was regulated by circulating factors released in proportion to adipose tissue mass that acted in the brain to maintain energy balance (92). While there was a concerted effort by researchers to identify circulating factors involved in energy homeostasis, it wasn't until 1994, when Zhang and colleagues successfully identified and cloned the *ob* gene and demonstrated that it is expressed selectively in adipose tissue and encodes a peptide hormone that was subsequently termed "leptin", mutation of which caused obesity in the *ob/ob* mouse (212). Not long thereafter, the leptin receptor was cloned and its expression demonstrated in hypothalamus and other tissues (183), and the *db* mutation was localized to this gene locus (100). As expected, either intraperitoneal or intracerebroventricular (i.c.v.) administration of leptin, reduced food intake and body weight in *ob/ob* mice, whereas *db/db* mice failed to respond (30, 76, 141, 192). Taken together, these data provided compelling initial support for the hypothesis that an adipose-tissue derived circulating factor acts in the CNS to regulate energy balance, and that maintenance of normal body fat stores is dependent on the integrity of this endocrine system. The clinical relevance of this work was confirmed by evidence that severe obesity results in humans as well as in mouse models from mutation of genes encoding either leptin (118, 212) or its receptor (100, 110, 181).

## B. Adiposity Signals: Insulin and Leptin

Several lines of evidence support the hypothesis that in addition to leptin, the pancreatic hormone insulin also acts in the brain as an "adiposity negative feedback signal". Both hormones circulate at levels that vary in proportion to body fat stores (4, 39) and both enter the CNS in proportion to their plasma level (160), where they act on their respective receptors expressed in key brain areas that control energy balance, glucose metabolism and autonomic function (10, 11, 53). Administration of either peptide into the brain reduces food

intake and body weight (30, 76, 200) while conversely, deficiency of either hormone or their respective receptors in the CNS results in hyperphagia, weight gain and insulin resistance (27, 174, 183, 212). In humans subjected to weight loss due to energy restriction, administration of leptin at doses that maintain pre-weight loss plasma levels blocks the reduction of sympathetic nervous system (SNS) activity and metabolic rate characteristic of the weight-reduced state (151). Taken together, these data support the hypothesis that energy homeostasis involves negative feedback signals that circulate in proportion to body fat and act centrally to inhibit food intake on the one hand, and to increase energy expenditure on the other. Although this review focuses primarily on leptin, its many areas of overlap with the actions of insulin in the brain warrant a focus on both hormones.

## II. LEPTIN SIGNALING

### A. Jak-STAT pathway

The leptin receptor contains an extracellular ligand-binding domain, a single transmembrane domain and a cytoplasmic signaling domain and is member of the interleukin (IL)-6 receptor family of class 1 cytokine receptors (182). Among the various isoforms of LepR, only the “long”, or “signaling” form, LepRb, appears to be competent for intracellular signal transduction and is critical for energy homeostasis. This assertion is based in part on evidence that mice that lack only LepRb (e.g. *db/db* mice) exhibit a phenotype similar to leptin-deficient *ob/ob* mice (100). Upon leptin binding to LepRb, the tyrosine kinase Janus kinase-2 (Jak2) is activated (69), resulting in the phosphorylation of three tyrosine residues on LepRb (Tyr<sub>985</sub>, Tyr<sub>1077</sub>, Tyr<sub>1138</sub>) that in turn recruit and activate a distinct set of downstream signaling proteins. Phosphorylation of Tyr<sub>985</sub> recruits SH2-domain-containing phosphatase-2 (SHP-2) to LepRb, leading to the activation of the extracellular signal regulated (ERK) signaling pathway in cultured cells, as well as the recruitment of suppressor of cytokine signaling-3 (SOCS-3), a negative regulator of LepRb signaling (6, 21, 22). In contrast, phosphorylation of Tyr<sub>1138</sub> recruits signal transduction and activator of transcription-3 (STAT3), which is then itself phosphorylated on tyrosine residues by Jak2 allowing it to dimerize and translocate to the nucleus where it functions as a transcription factor (6, 23, 186). Among the genes transcriptionally induced by STAT3 is SOCS-3, which causes negative feedback inhibition of leptin signaling by binding to Tyr<sub>985</sub> of LepRb to block its signaling (Figure 2). Phosphorylation of Tyr<sub>1077</sub> and Tyr<sub>1138</sub> promote the recruitment, phosphorylation and transcriptional activation of STAT5 (71), and leptin may also activate signals generated by Jak2 phosphorylation that are independent of tyrosine phosphorylation sites on LepRb.

To determine the functional significance of each of these LepRb tyrosine residues *in vivo*, an approach was taken to generate homologously targeted “knock-in” mice whereby sequences encoding substitution mutants of specific LepRb phosphorylation sites replace the endogenous *LepR* allele. Mice bearing a mutant LepRb gene in which Tyr<sub>1138</sub> is replaced by a serine residue (*s/s* mice) are unable to recruit STAT3 and are characterized by hyperphagia, obesity (14) and reduced energy expenditure (12), suggesting that the Tyr<sub>1138</sub> → STAT3 pathway plays a crucial role in the regulation of energy homeostasis (12, 14). However, the phenotype of these *s/s* mice does not completely mimic that of either *ob/ob* or *db/db* mice with respect to glucose metabolism, immune function or reproduction (13, 14), implying that other LepRb signals must also contribute to leptin action.

To determine the contribution of LepRb Tyr<sub>985</sub> to leptin action *in vivo*, mice were generated in which the endogenous LepRb allele was replaced with a mutant in which a serine residue is substituted for Tyr<sub>985</sub>. In contrast to the hyperphagic, obese phenotype of animals mutant for Tyr<sub>1138</sub>, mutation of Tyr<sub>985</sub> in these (*l/l*) mice resulted in a lean phenotype characterized by reduced food intake and adiposity, and increased leptin sensitivity (24). These results

support data from cultured cells (22) suggesting that Tyr<sub>985</sub> attenuates leptin action *in vivo*, presumably by serving as the SOCS-3 binding site (24). Since SOCS-3 can also be induced via the IKK $\beta$ -NF $\kappa$  $\beta$  pathway during cellular inflammation, this mechanism can contribute to leptin resistance in this setting (211) and has been implicated in the pathogenesis of diet-induced obesity (DIO) (87, 119, 211). What remains uncertain based on the phenotype of both *s/s* and *l/l* mice is the extent to which Tyr<sub>1077</sub> and/or Jak2-dependent signals independent of LepRb tyrosine phosphorylation mediate leptin action *in vivo*.

Recent evidence suggests that leptin is capable of activating STAT5 in the hypothalamic arcuate nucleus (ARC) *in vivo* (126), in addition to cultured cells (71), raising the possibility that LepRb $\rightarrow$ STAT5 signaling contributes to the physiological actions of leptin. This hypothesis is supported by evidence that neuronal deletion of STAT5 in mice (via *RIP-cre* or *Nestin-cre*) causes a mild-obesity phenotype (101). However, in addition to leptin, several other cytokines and growth factors activate STAT5 including granulocyte macrophage colony stimulating factor (GM-CSF), prolactin and growth hormone, amongst others (101). Second, the use of *RIP-cre* and *Nestin-cre* deletes some, but not all LepRb-expressing neurons as well as deleting many non-LepRb-expressing neurons. Generation of mice bearing LepRb Tyr<sub>1077</sub> substitution will help to clarify the physiological role of leptin-mediated STAT5 signaling.

To investigate the potential role in leptin action for signals activated by Jak2 independent of LepRb phosphorylation, homologous recombination was used to replace *Lepr* with a truncated LepRb mutant (LepRb $\Delta^{65}$ ) (150). These  $\Delta/\Delta$  mice exhibit a phenotype that resembles *db/db* mice where energy homeostasis, neuroendocrine and immune function are concerned, but also are characterized by a delayed progression to diabetes, suggesting a modest improvement in glucose homeostasis (150). Taken together, these findings suggest that Jak2-autonomous LepRb signals are insufficient to mediate most of the physiological actions of leptin.

Studies from knock-in mice have therefore offered important insight into the physiological contribution of specific tyrosine residues on LepRb to leptin action *in vivo*. Although leptin clearly activates the Jak-STAT pathway in mediobasal hypothalamus (186) and although this pathway is clearly critical for leptin regulation of energy homeostasis (14), leptin also elicits neuronal responses in a time frame of seconds to minutes that cannot be explained by STAT3-mediated changes of gene transcription. For instance, in electrophysiological studies using freshly prepared hypothalamic slices, leptin rapidly changes the membrane potential and firing rate of neurons (42, 176), and leptin-induced inhibition of food intake is observed within an hour. These observations prompted a search for leptin-activated signal transduction mechanisms additional to the Jak-STAT pathway.

## B. IRS-PI3K pathway

Similarities between insulin and leptin action in the CNS prompted investigation of the hypothesis that intracellular signaling by insulin and leptin converge in key neuronal subsets at the level of the insulin receptor substrate-phosphatidylinositol-3-OH kinase (IRS-PI3K) pathway (Figure 3). Initial support for this hypothesis was provided by electrophysiological studies in which leptin and insulin were shown to hyperpolarize a subset of “glucose-responsive” neurons in the ARC via activation of the ATP-sensitive potassium channel (K<sub>ATP</sub>) (176, 177) via a mechanism that was blocked by a PI3K inhibitor (116, 177). Other early studies showed that like insulin, leptin activates the IRS-PI3K pathway in the hypothalamus, and pharmacological inhibition of PI3K activity in the brain blocks the ability of leptin to reduce food intake (129, 130). A potential mechanism linking the leptin receptor to IRS-PI3K signaling involves the protein SH2B1 (103), which appears to facilitate Jak2-mediated IRS phosphorylation in response to leptin receptor activation.

Interesting, genome-wide scans have identified the SH2B1 gene locus as an obesity risk allele in human populations (195). Leptin has also been reported to activate nutrient-sensing enzymes including AMP-activated protein kinase (AMPK) (115) and mammalian target of rapamycin (mTOR) signaling pathways (41), although neither the neuronal subsets nor the molecular mechanism involved has been elucidated.

We note here that although leptin and insulin both have the potential to activate IRS/PI3K signaling in neurons and other cell types, both the subcellular localization and intracellular consequences of this activation can differ substantially depending on cell type and on whether activation is mediated by leptin vs. insulin. Stated differently, the assertion that similarities in the behavioral or metabolic actions of insulin and leptin in the brain reflect convergence of intracellular signaling via PI3K or other transduction mechanisms is an oversimplification, and additional studies are needed to better understand neuronal responses to these hormones and their consequences for energy homeostasis and glucose metabolism (157). Progress made using mouse genetics in this effort is discussed below under Leptin-Sensitive Neuronal Subsets.

### III. CNS-MEDIATED EFFECTS OF LEPTIN ON GLUCOSE METABOLISM

#### A. Leptin and Glucose Metabolism

In addition to the well-established role played by leptin in the regulation of energy homeostasis (198), growing evidence implicates leptin in glucose homeostasis as well, particularly in the control of peripheral tissue insulin sensitivity. The earliest evidence for such a role comes from studies in mice with genetic leptin deficiency (*ob/ob* mouse) or leptin receptor deficiency (*db/db* mouse or *fa/fa* rat). Such animals exhibit not only hyperphagia and obesity, but insulin resistance and diabetes as well (37, 57, 181, 212). While increased food intake and body adiposity clearly contribute to impaired glucose metabolism in these rodent models, several observations suggest that leptin regulates glucose metabolism independently of its effects on energy balance. Not only is caloric restriction limited in its ability to improve insulin sensitivity and hyperglycemia in animals with genetic leptin or leptin-receptor deficiency (122, 203), leptin administration to leptin-deficient mice or humans ameliorates hyperglycemia and hyperinsulinemia (60, 141) even when differences of food intake are controlled by pair-feeding (80, 158).

Severe insulin resistance and diabetes are also observed in genetic disorders characterized by reduced leptin levels due to disorders of adipose tissue development. Such conditions, termed “lipodystrophy,” can result from any of several mutations that impair adipogenesis and thus severely limit the capacity to store triglyceride. In humans, the two most common genetic lipodystrophies are congenital generalized lipodystrophy (CGL), a condition in which body fat is absent from birth, and familial partial lipodystrophy (FPL), where the loss of body fat is progressive and variable, occurring during childhood and puberty (172). In addition to genetic lipodystrophy, acquired lipodystrophies also have been described (and are more common), including acquired partial lipodystrophy (APL), acquired generalized lipodystrophy (AGL), and that induced by highly active antiretroviral therapy (HAART) containing HIV-1 protease inhibitors in HIV-infected patients.

A pathophysiological sequence of events shared in common by these lipodystrophic disorders is that the loss of adipocytes and the inability to store fat leads to accumulation of excess nutrients (including triglyceride) in liver, muscle and other tissues, causing metabolic impairment. Several lines of evidence from both humans and rodent models suggest that leptin deficiency contributes to insulin resistance and diabetes in this setting (139, 168, 169). For one, transplantation of body fat from wild-type animals improves insulin sensitivity in a mouse model of lipodystrophy, whereas transplantation of fat from *ob/ob* mice has no effect

(38, 63). As in leptin-deficient mice, the diabetes phenotype of these lipodystrophic mice is also ameliorated by leptin administration via a mechanism that is, at least in part, independent of its effect on food intake and body weight (64, 168). In humans, leptin therapy has been examined in several clinical trials in patients with lipodystrophy and these have demonstrated significant improvements in glycemia, dyslipidemia and hepatic steatosis (89, 90, 139, 142). At present, congenital lipodystrophy is the only clinical condition for which a compelling indication for leptin therapy has been established.

Another model of leptin deficiency is that which occurs in uncontrolled insulin-deficient diabetes (uDM), induced by the loss of insulin-secreting beta-cells. As the synthesis and storage of fat requires insulin, weight gain cannot occur in uDM, despite elevated food intake, known as “diabetic hyperphagia,” in this setting. Consequently, uDM is characterized by both hyperglycemia and hyperphagia (102), with progressive loss of body fat stores, and with a dramatic reduction of circulating levels of leptin as well as insulin (78). Since the excess calories consumed cannot be stored as fat, they contribute to hyperglycemia and are ultimately lost through the urine, and the ensuing diuresis causes dehydration leading to increased water intake (108). Although insulin deficiency is primarily responsible for hyperglycemia and weight loss in uDM, at least some of the beneficial effects of insulin treatment may in fact arise from an associated increase of leptin action since plasma leptin levels are normalized by insulin treatment in uDM rats (78). Similarly, reduction in the plasma level of both leptin and insulin is implicated in the pathogenesis of diabetic hyperphagia in rats treated with streptozotocin (STZ) (173), a chemical agent that selectively destroys pancreatic beta cells. A role for leptin deficiency in the hyperphagia characteristic of uDM was established by preventing the fall in plasma leptin levels by systemic administration of exogenous leptin at a dose that maintains normal physiological plasma leptin levels in rats with STZ-induced diabetes (173).

Similarly, leptin deficiency has recently been implicated in the progressive insulin resistance and associated neuroendocrine derangements seen in uDM. Systemic administration of exogenous leptin at a dose that maintains normal physiological plasma leptin levels prevented the development of severe, progressive insulin resistance in rats with uDM, an effect that could not be explained by leptin-induced changes of food intake or body weight (68). Moreover, the mechanism underlying this effect appeared to preferentially involve the liver, as physiological leptin replacement in uDM reduced hepatic triglyceride content and gluconeogenic gene expression, and also restored normal insulin signal transduction in liver, but not in skeletal muscle or WAT. This intervention also normalized circulating levels of glucagon and corticosterone, both of which are elevated in uDM. Thus, leptin deficiency plays a key role in neuroendocrine disturbances linked to hyperglycemia in uDM. Yet physiological leptin replacement only modestly reduced blood glucose levels in this setting (68), demonstrating that factors beyond leptin deficiency (and elevated levels of glucagon and corticosterone) are sufficient to explain hyperglycemia in this model. Thus, although prevention of leptin deficiency may ameliorate insulin resistance and related endocrine disorders and hence confer benefit to humans with diabetes, this intervention seems unlikely to substantially improve hyperglycemia in and of itself.

Interestingly, hyperleptinemia induced by either pharmacological leptin administration (34) or with adenoviral gene therapy (210) fully ameliorates hyperglycemia in STZ-induced diabetic rats, even in the face of extremely low plasma insulin levels. Although the mechanism underlying this effect requires additional study, it may involve a suppression of hepatic glucose production (HGP). In normal individuals, insulin-induced suppression of HGP is critical for the control of blood glucose levels. Consequently, in uDM, glucose production is unrestrained, and therefore contributes importantly to diabetic hyperglycemia. Several lines of evidence suggest that hyperglucagonemia contributes to the hyperglycemia

in uDM, at least in part by driving the hepatic expression of the gluconeogenic genes, glucose-6-phosphatase (*G6Pase*) and phosphoenolpyruvate kinase (*Pepck*) (47, 123).

This hypothesis was articulated by Yu, et al., who reported that the anti-diabetic effects of leptin in STZ-induced diabetes are accompanied by normalization of elevated glucagon levels (210). Moreover, glucagon receptor-null mice remain normoglycemic following treatment with alloxan to induce uDM, suggesting that intact glucagon signaling is required for diabetic hyperglycemia and hence that the leptin-mediated decrease of plasma glucagon levels may mediate its effects on blood glucose (191). Although this hypothesis merits further study, the observation that in rats with STZ-induced uDM, hyperglycemia is only slightly improved by physiological leptin replacement despite normalized plasma glucagon levels (68) suggests that the latter cannot fully explain the hypoglycemic effect of pharmacological leptin administration in this setting. Collectively, these data suggest that deficient endogenous leptin signaling underlies at least some manifestations of uDM, and raise the intriguing possibility that leptin therapy may one day constitute a useful adjunct to insulin in diabetes treatment.

## B. Sites and CNS Mechanisms Underlying Leptin Action on Glucose Metabolism

To determine the mechanism by which leptin influences glucose metabolism, the acute effects of a pharmacological dose of leptin on peripheral and hepatic insulin action were studied using the euglycemic-hyperinsulinemic clamp technique. In non-diabetic rats, an acute intravenous (i.v.) leptin infusion enhanced insulin's ability to suppress glucose production without affecting glucose uptake. This enhanced insulin-mediated suppression of glucose production was due to a marked suppression of hepatic glycogenolysis, whereas gluconeogenesis was paradoxically increased (152). In wild-type mice, i.v. leptin increased glucose turnover and glucose uptake but decreased hepatic glycogen content (91). In *ob/ob* mice, i.v. leptin also increased glucose turnover and stimulated glucose uptake in brown adipose tissue (BAT), brain and heart, but not in skeletal muscle or white adipose tissue (WAT) (29). Among several lines of evidence implicating the brain in the actions of leptin on glucose metabolism is the observation that the effect is similar whether leptin is administered i.v. or via i.c.v. infusion (at much lower doses) in both rats (106) and mice (91). Despite clear evidence that leptin has direct actions on pancreatic  $\beta$ -cells, skeletal muscle, fat, and other tissues, low-dose i.c.v. administration of leptin corrects the insulin resistance and diabetes phenotype of both leptin deficient *ob/ob* and lipodystrophic mice (2). Similarly, i.c.v. administration of leptin normalizes blood glucose levels in STZ-diabetic rats at doses that are ineffective when administered peripherally (43, 82, 104, 190). Moreover, we recently found that continuous infusion of leptin directly into the brain normalizes blood glucose levels in STZ-induced diabetic rats via a mechanism that cannot be explained by reduced food intake, increased urinary glucose losses or a recovery of pancreatic  $\beta$ -cells. Instead, leptin action in the brain potently suppressed hepatic glucose production while increasing tissue glucose uptake despite persistent, severe insulin deficiency (67). This leptin action is distinct from its previously reported effect to increase insulin sensitivity in the liver (66, 146), and offers compelling evidence of a novel mechanism through which the brain normalizes diabetic hyperglycemia without the need for insulin (67). Taken together, these data provide compelling evidence that the CNS mediates key effects of leptin on glucose metabolism.

Using in situ hybridization and immunohistochemical techniques, *lepr<sup>b</sup>* mRNA and protein have both been detected in several hypothalamic areas involved in the control of energy balance and autonomic function, including the ARC, ventromedial nucleus (VMH) and dorsomedial nucleus (DMN), with lesser amounts in the paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA) (10, 53). Beyond the hypothalamus, functional leptin receptors are expressed in the hippocampus, hindbrain (including the nucleus of the solitary

tract (NTS)), pyriform cortex and other olfactory processing areas, and in mesolimbic areas involved in reward including the ventral tegmental area (VTA) and substantia nigra (SN) (61, 72).

To investigate the role of the ARC in the action of leptin on peripheral glucose metabolism, gene targeting and gene therapy techniques have been used to selectively restore leptin signaling to this brain area in leptin receptor-deficient animals. Coppari and colleagues studied mice in which the endogenous *Lepr* allele was replaced by a mutant construct through which gene expression is blocked by insertion of a “floxed” stop cassette (*Lepr<sup>neo/neo</sup>* mice). Microinjection of a virus expressing cre recombinase was then used in these mice to restore *Lepr* expression in a brain region-specific manner. With this approach, unilateral, selective restoration of leptin signaling to the ARC was found to have only modest effects on food intake and body weight, while it markedly improved hyperinsulinemia and normalized blood glucose levels (40). We developed a complementary approach using the leptin receptor-deficient Koletsky (*fa<sup>k</sup>/fa<sup>k</sup>*) rat, in which genetic obesity arises from a point mutation that results in the absence of all forms of the leptin receptor (181). These animals are characterized by obesity, hyperphagia, insulin resistance and glucose intolerance but are not hyperglycemic (57). Using adenoviral gene therapy, we found that selective restoration of leptin receptor signaling to the ARC significantly improved peripheral insulin sensitivity via a mechanism that was not dependent on reduced food intake or body weight (122). Furthermore, the effect of leptin signaling in the ARC to improve insulin sensitivity was attenuated by i.c.v. administration of the PI3K inhibitor, LY294002, while it was mimicked by ARC-directed expression of a constitutively active mutant of Akt/protein kinase B (PKB), a key downstream mediator of PI3K (122). These findings suggest that leptin signaling limited to the ARC is sufficient to promote normal insulin sensitivity, and that the neuronal signaling mechanism underlying this leptin effect involves PI3K.

### C. Role of Hypothalamic PI3K – Insight from CNS Insulin Action

The hypothesis that PI3K signaling contributes to the effects of hypothalamic leptin action on glucose metabolism is further supported by studies in which the endogenous leptin receptor was replaced with a mutant allele (*lepr<sup>S1138</sup>*) that prevents LepRb-STAT3 signaling without affecting other transduction pathways. As expected, these mice exhibit hyperphagia and obesity, yet their impairment of glucose metabolism is mild compared to either *ob/ob* or *db/db* mice (13). Furthermore, since defective glucose metabolism in these mice is prevented by chronic caloric restriction (13), whereas this does not occur in calorically-restricted *db/db* mice (203), leptin receptor-mediated STAT3 signaling appears to be critical for energy homeostasis, but not for glucose homeostasis. Leptin-stimulated PI3K signaling likely contributes to this mechanism, based on findings noted above (122), and is consistent with recent evidence that Jak2-mediated signals that are independent of LepRb tyrosine phosphorylation contribute to the modulation of glucose homeostasis by leptin (150).

Like leptin, insulin also activates the IRS-PI3K pathway in hypothalamic neurons, and PI3K is also required for the ability of insulin to inhibit food intake (129). While insulin is well known for its actions in peripheral tissues such as liver, muscle and fat in the control glucose metabolism, several studies identify a role for insulin action in the brain as well. For example, mice with neuron-specific deletion of either the insulin receptor (IR) or insulin receptor-substrate-2 (IRS-2) are characterized by mild obesity and insulin resistance (27, 97, 105). Moreover, while selectively restoring insulin receptors to the liver and pancreas of insulin receptor-deficient mice rescues them from perinatal lethality, restoration of insulin signaling to the brain as well prevents the development of diabetes (136).



Using a complementary approach, Koch and colleagues acutely inactivated insulin receptor signaling either in all tissues including brain ( $IR^{\Delta wb}$ ) or in a manner that was restricted to peripheral tissues ( $IR^{\Delta per}$ ). The  $IR^{\Delta per}$  mouse model used cre-recombinase fused to the estrogen receptor to acutely induce insulin receptor-deficiency in peripheral tissues, through peripheral administration of the synthetic estrogen receptor antagonist, tamoxifen. To acutely induce insulin receptor-deficiency in both peripheral tissues and brain, a second mouse model was created using conditional RNAi-mediated gene knockdown through the expression of a ubiquitous IR-specific shRNA under the transcriptional control of the human H1/U6 promoter that contains a tetracycline-operated sequence (tetO). Upon doxycycline administration, the tetracycline repressor (tetR) which silences the shRNA is sequestered from the promoter, allowing ubiquitous transcription of the IR shRNA. Using each of these elegant approaches, inducible inactivation of the IR in both peripheral tissues and brain consistently causes a more pronounced diabetes phenotype than when receptor deletion occurs in peripheral tissues alone, thus implicating neuronal insulin action in the control of glucose homeostasis (95).

Consistent with a role for CNS insulin action in the control of glucose metabolism, either intrahypothalamic administration of antisense oligonucleotides to reduce insulin receptor signaling or local infusion of the PI3K inhibitor, LY294002, causes hepatic insulin resistance in rats (132). Conversely, administration of insulin into either the third ventricle or the mediobasal hypothalamus improves insulin sensitivity via enhanced suppression of hepatic glucose production, with no effect on glucose uptake (135). Moreover, these effects of insulin are blocked by infusion of the PI3K inhibitor and also appear to require activation of  $K_{ATP}$  channels (135, 145). Similarly, increasing hypothalamic PI3K signaling, a major intracellular mediator of insulin action, by local overexpression of IRS-2 increases peripheral insulin sensitivity in rats with uDM induced by STZ (65). Collectively, these studies suggest that neuronal PI3K signaling is an important determinant of glucose metabolism, and that the CNS action of insulin is mediated via this mechanism.

#### D. CNS Nutrient-Sensing

In addition to insulin and leptin, the CNS is capable of sensing and responding to input from nutrient-related signals. Several lines of evidence suggest that one hypothalamic signal of nutrient availability is the intracellular accumulation of long-chain fatty acyl-CoA (LCFA) molecules. Consistent with this hypothesis, pharmacological interventions designed to block LCFA oxidation by increasing malonyl CoA levels (e.g. oleic acid, fatty acid synthase (FAS) inhibitor) in the CNS reduce food intake and HGP (99, 107, 133, 147), while conversely, pharmacological interventions that reduce hypothalamic malonyl CoA levels cause obesity and insulin resistance (79). Nutrient-sensing involving in the CNS play an important role in the regulation of energy balance and glucose metabolism and are described in greater detail elsewhere (31, 98).

The enzyme AMP-activated protein kinase (AMPK) is a ubiquitous fuel sensor that is activated when cellular fuel availability is low. AMPK phosphorylates and inhibits acetyl Co-A carboxylase (ACC), preventing the conversion of acetyl CoA to malonyl CoA, leading to a fall in malonyl CoA levels (179). This, in turn, increases energy utilization (e.g. fat oxidation) by disinhibiting the enzyme carnitine palmitoyltransferase-1 (CPT-1) (154). In the hypothalamus, AMPK activity is sensitive not only to changes of nutrient (e.g., glucose) availability, but is inhibited by central administration of either insulin or leptin (115, 208). Further, inhibition of hypothalamic AMPK (using an adenovirus expressing the dominant negative form of AMPK) lowers food intake and HGP, while increasing hypothalamic AMPK has the opposite effect (113, 209), mimicking some hypothalamic actions of leptin and insulin. These findings collectively implicate cellular nutrient-sensing mechanisms as downstream mediators of at least some leptin and insulin effects.

## E. Leptin-Sensitive Neuronal Subsets

Since its discovery, the neuronal mechanism(s) by which leptin acts in the brain has been an area of intense focus. Peripheral administration of leptin activates neuronal populations throughout the brain, as measured either by pSTAT3 activation (a marker of LepRb activation) or induction of c-fos expression (which can occur both in “first order” neurons activated directly by leptin and in “second order” neurons in a leptin-activated circuit), including regions throughout the hypothalamus such as the ARC, VMH, PVN, LHA and dorsomedial hypothalamus (DMH), and extrahypothalamic areas including the VTA, NTS and thalamus, amongst others (50, 52, 186). Ongoing studies have employed mouse genetics and other strategies to determine the functional role of each of these leptin-sensitive neuronal subsets in the actions of leptin on glucose metabolism, energy balance, immune function and reproduction (128).

Two of the most well characterized leptin-sensitive neuronal populations implicated in the regulation of food intake, energy expenditure and glucose metabolism are contained within the hypothalamic ARC. One of these subsets expresses neuropeptide Y (NPY) and agouti related peptide (AgRP), peptides that stimulate food intake and inhibit energy expenditure, thereby promoting positive energy balance (75, 153, 178). As these NPY/AgRP neurons are inhibited by both leptin and insulin, reduced neuronal input from these hormones (as occurs, for example, during fasting and uDM) increases hypothalamic expression of these peptides (158, 163).

Adjacent to these cells is a neuronal subset that expresses pro-opiomelanocortin (POMC). Unlike NPY/AgRP neurons, POMC neurons are stimulated by leptin (33, 162) whereas the effects of insulin are more complex and context-dependent (35, 204). The POMC cleavage product, alpha-melanocyte stimulating hormone ( $\alpha$ -MSH) is released by these neurons and acts on melanocortin receptors (Mc3r/Mc4r) to inhibit food intake, increase energy expenditure and promote weight loss (59). Therefore, in conditions of reduced leptin signaling (e.g. fasting or diet-induced weight loss), POMC neurons are inhibited while NPY/AgRP neurons are activated, a combination of responses that promote positive energy balance and the recovery of lost weight. Hence, NPY/AgRP neurons stimulate feeding not only by releasing NPY, and thereby activating Y1 and Y5 receptors, but by reducing melanocortin signaling via release of AgRP, an endogenous melanocortin-3/4 receptor antagonist (137, 171). Moreover, NPY/AgRP neurons inhibit POMC neurons through release of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) (42) and recent work suggests that loss of GABAergic signaling by AgRP neurons projecting to the parabrachial nucleus causes profound anorexia (201).

While each of these two neuronal subpopulations is critical for normal energy homeostasis, both also appear to be important in the regulation of glucose homeostasis. Central administration of both NPY and the melanocortin-3/4 receptor antagonist, SHU9119, cause insulin resistance and glucose intolerance via mechanisms independent of their effects on food intake (1, 109, 188). Conversely, chronic central administration of the melanocortin agonist, melanotan II (MTII) has the opposite effect (134).

Several additional studies have sought to clarify the role of leptin signaling via the Jak-STAT and the IRS-PI3K pathway in ARC neurons. Electrophysiological studies first demonstrated that both insulin and leptin activate ATP-sensitive potassium channels and thereby hyperpolarize a subset of “glucose-responsive” neurons via a mechanism that requires PI3K (176, 177). Consistent with this, leptin inhibits the firing rate of NPY/AgRP neurons (189), while simultaneously depolarizing POMC neurons (42), and also activates PI3K signaling in the latter cells (204). In contrast to leptin, however, insulin hyperpolarizes and silences both AgRP and POMC neurons (96) and activates PI3K signaling in both these

neuronal populations (204). In POMC neurons, therefore, both insulin and leptin activate PI3K signaling, but leptin leads to membrane depolarization and an increase in firing rate, whereas insulin evidently has the opposite effects. Complicating matters further, leptin reduces PI3K signaling in AgRP cells, whereas insulin increases it (204), and yet both hormones inhibit AgRP neuronal firing. Consequently, it has been difficult to reconcile how two different hormones share the same signaling cascade, yet have opposite effects on neuronal firing. To further clarify the role of various intracellular signaling transduction mechanisms in leptin action *in vivo*, Cre-LoxP technology has been utilized to delete leptin receptors, STAT3 or components of the IRS-PI3K pathway in each of these neuronal populations.

Mice with selective deletion or inactivation of either the insulin receptor or IRS-2 in POMC cells (35, 96) exhibit no obesity phenotype, unlike what is observed with pan-neuronal deletion of these proteins. When both subunits of PI3K (p85 $\alpha$ , p110 $\alpha$ ) were deleted from POMC neurons in mice, leptin failed to depolarize and increase the firing rate of these neurons, and insulin's ability to hyperpolarize POMC neurons was similarly abolished. However, while the anorexic effects of leptin were blunted in these mice, body weight was not altered (84). To further examine the role of the IRS-PI3K pathway in POMC neurons on energy and glucose homeostasis, studies were conducted in which signaling molecules downstream of PI3K were deleted selectively in these cells.

Following its activation, PI3K phosphorylates phosphatidyl-inositol-4,5-bisphosphate (PIP<sub>2</sub>) to phosphatidyl-inositol-3,4,5-triphosphate (PIP<sub>3</sub>) and activates several downstream molecules including 3-phosphoinositide-dependent protein kinase 1 (PDK1) and protein kinase B (PKB, also referred to as Akt) (Figure 3A). While selective inactivation of PDK1 in POMC neurons caused a mild-obesity phenotype (15), selective inactivation of the PIP<sub>3</sub> phosphatase, PTEN (phosphatase and tensin homologue) in these neurons unexpectedly caused a mild-obesity phenotype, despite increased PIP<sub>3</sub> signaling (143). Yet deletion of PTEN in all leptin receptor-expressing cells (e.g., POMC cells along with many other cell types) protects against DIO and improves insulin resistance during high-fat (HF) feeding (144). These seemingly contradictory findings remain incompletely explained, but may reflect multiple, divergent roles for PI3K signaling within a given cell subpopulation, and divergent effects of leptin *vs.* insulin arising due to differences in the intracellular microdomain in which PI3K signaling is activated. Thus, while it has been established that anorexia induced by icv leptin can be blocked by inhibiting PI3K signaling using a pharmacological approach (130), the contribution of the PI3K signaling pathway in specific hypothalamic neurons to leptin regulation of energy balance and glucose metabolism has been less clear.

To address this conundrum, Elmquist and colleagues recently provided both histological and electrophysiological evidence that leptin and insulin act in distinct subpopulations of POMC cells, rather than acting on the same subset of POMC cells (197). This conclusion is based on evidence that the subset of POMC cells in which leptin-induced *c-fos* activity is detected is separate from those POMC neurons that express the insulin receptor, and they confirmed that acute effects of insulin in POMC cells differs from that of leptin in ways that suggest an anatomical separation of insulin- and leptin-responsive POMC cells (197). These data support a model in which "crosstalk" between insulin and leptin involves distinct subpopulation of POMC cells, rather convergent signal transduction in the same neurons (Figure 3A,B).

Taken together, these findings offer a plausible explanation for how both insulin and leptin activate the same intracellular signaling enzyme -- PI3K -- and yet have divergent effects on POMC neuronal activity. To further clarify the physiological roles of insulin and leptin

signaling in POMC neurons, mice lacking both leptin and insulin receptors in POMC neurons (*Pomc-Cre, Lepr<sup>flox/flox</sup> IR<sup>flox/flox</sup>* mice) were compared to mice lacking either insulin receptors alone or leptin receptors alone in these cells (83). Consistent with previous reports, body weight was not affected by deletion of insulin receptors alone in POMC neurons (96), whereas mice lacking leptin receptors from POMC neurons exhibit a mild-obesity phenotype (5). However, the combined deletion of insulin receptors and leptin receptors in POMC neurons ameliorated the obesity phenotype of mice lacking leptin receptors from POMC neurons (83). While previous studies had reported either no effect (5) or only a mild effect on glucose homeostasis in male mice lacking leptin receptors in POMC neurons (165), female mice lacking both insulin- and leptin-receptors in POMC neurons exhibited marked hepatic insulin resistance, as measured using the euglycemic-hyperinsulinemic clamp technique (83), suggesting that actions of both insulin and leptin on discrete subpopulations of POMC neurons act synergistically to suppress HGP.

While leptin-induced activation of STAT3 is critical for normal energy homeostasis (12, 14), selective deletion of STAT3 in either POMC (205) or NPY/AgRP (70) neurons results in a mild-obesity phenotype, as occurs when leptin receptors are selectively deleted from these cells (5, 46, 187). Conversely, overexpression of STAT3 in AgRP neurons results in a lean phenotype that is resistant to DIO due to an increase in locomotor activity and energy expenditure (112). Deletion of leptin receptors from both NPY/AgRP and POMC ARC neurons results in a phenotype that is nearly additive with respect to the increase of body weight and adiposity observed following deletion of leptin receptors from individual neuronal subsets, but the overall effect is still modest compared to obesity caused by leptin deficiency (187). This outcome is not surprising, given that leptin receptors are expressed in many other hypothalamic areas, as well as in extrahypothalamic sites that likely contribute to leptin's anorectic effects. Consequently, leptin receptors must be restored widely throughout the CNS using several neuron-specific promoters to normalize obesity, fertility and glucose metabolism in *db/db* mice (44).

The impact of leptin signaling in POMC neurons on energy homeostasis and glucose metabolism was also recently investigated by Bjorbaek and colleagues by reactivating leptin signaling selectively in POMC neurons of mice that otherwise lack leptin receptors. Expression of leptin receptors selectively in POMC neurons of *Lepr<sup>db/db</sup>* mice (*HA-LRb/Pomc-Cre/Lepr<sup>db/db</sup>*) reduced food intake and body weight, while normalizing blood glucose levels independently of changes in energy balance (88). These data provide further evidence that leptin signaling in POMC neurons plays an important role in leptin's ability to regulate glucose metabolism. Similarly, while mice with selective inactivation of the insulin receptor in AgRP neurons maintain normal body weight, they exhibit hepatic insulin resistance, implying a role for insulin action in AgRP expressing cells in the maintenance of normal liver insulin sensitivity (96).

In addition to leptin action in ARC neurons, several lines of evidence suggest that the VMH also plays an important role in leptin's effects on glucose metabolism. Neurons in the VMH express the leptin receptor (53), are activated by leptin (as measured by induction of either pSTAT3 or cFos) (124), and leptin rapidly depolarizes and increases the firing rate of steroidogenic factor-1 (SF1)-containing neurons in this brain area (180). In addition, microinjection of leptin directly into the VMH promotes glucose uptake in peripheral tissues via a mechanism involving the SNS (77, 114), whereas selective deletion of leptin receptors from SF-1 positive neurons results in an obese, insulin-resistant phenotype, an effect that is exaggerated when leptin receptor deficiency is present in both POMC and VMH neurons (20, 46). Neurons in the VMH are implicated in the regulation of melanocortin signaling (180), whereas melanocortins also regulate the function of VMH neurons. For example, BDNF-containing neurons in the VMH are regulated by both nutritional state and by Mc4r

signaling (206). Furthermore, mice with reduced expression of the BDNF receptor, TrkB, exhibit a phenotype characterized by obesity, hyperphagia and increased body length, similar to that of Mc4r deficient mice, and leptin administration induces *Bdnf* expression in the VMH (206). Given that a subset of VMH neurons send strong excitatory inputs to POMC neurons in the ARC (180), these data implicate a subset of VMH neurons as components of the melanocortin pathway and suggest that leptin signaling in this brain area participates in the control of glucose metabolism.

The VMH is also implicated in the control of glucagon secretion. Studies dating back to the 1960's and 1970's demonstrated that electric stimulation of the VMH increases glucagon secretion, promotes liver glycogen breakdown and raises blood glucose levels via the ANS (166, 167). Moreover, VMH neurons are critical for sensing and responding to hypoglycemia (111). Thus, leptin signaling in the VMH offers a plausible mechanism to explain leptin-mediated normalization of hyperglycemia and hyperglucagonemia in STZ-induced diabetes (210).

## F. Effect of CNS Leptin to Regulate Glucose Metabolism

Investigation into how leptin signaling in the brain improves peripheral insulin sensitivity has been hampered to some extent by divergent outcomes from studies that employed a pharmacological approach in normal animals versus those using a gene therapy approach in animals that lack leptin receptors. In normal rats, i.c.v. leptin administration stimulates hepatic gluconeogenesis, but fails to affect overall hepatic glucose production owing to a simultaneous decrease of glycogenolysis (106). Subsequent studies demonstrated that the effect of i.c.v. leptin on gluconeogenesis and expression of the gluconeogenic enzyme, *Pepck* were blocked when activation of neuronal melanocortin signaling was inhibited using the melanocortin-3/4-receptor antagonist, SHU9119, while leptin suppression of glycogenolysis remained intact, resulting in a net decrease of glucose production (73). Thus, pharmacological leptin stimulation of gluconeogenesis appears to involve a melanocortin-dependent pathway while leptin inhibition of glycogenolysis is proposed to occur via a melanocortin-independent pathway. In addition, the acute effects of hypothalamic leptin signaling on liver glucose flux require leptin receptor signaling through STAT3 (28). Since i.c.v. leptin infusion reverses insulin resistance induced in rats by short-term exposure to a HF diet by reducing both glycogenolysis and gluconeogenesis (146), these data suggest that that metabolic response of normal rats to i.c.v. leptin is context-dependent, and further, that CNS responses may contribute to the pathogenesis of peripheral insulin resistance during HF feeding.

Recent work has begun to clarify how hypothalamic leptin action affects peripheral glucose metabolism. Using the euglycemic-hyperinsulinemic clamp technique to measure changes of glucose metabolism induced by restoring leptin receptor signaling to the ARC of leptin receptor-deficient Koletsky rats, improved insulin sensitivity was shown to involve enhanced insulin-induced suppression of glucose production, rather than changes of glucose uptake (66). These effects of restored ARC leptin signaling were associated with enhanced insulin signal transduction via PI3K in liver, but not skeletal muscle and were also associated with reduced hepatic expression of key gluconeogenic genes, *G6Pase* and *Pepck* (66). Thus, leptin action in the ARC increases peripheral insulin sensitivity primarily via effects on the liver, and like insulin, this effect is PI3K-dependent and involves inhibition of hepatic gluconeogenic gene expression.

One potential mechanism whereby leptin could alter hepatic insulin sensitivity is via inhibition of the hepatic enzyme, stearoyl-coenzyme A desaturase 1 (SCD-1), which catalyzes the synthesis of monounsaturated fatty acids (MUFA, such as oleic acid) from saturated FFA (e.g. palmitate, stearate) and is essential for de novo lipogenesis. Hepatic

*Scd-1* mRNA levels are markedly increased in *ob/ob* mice, an effect implicated in the dramatic increase of hepatic triglyceride synthesis and steatosis characteristic of these animals (36). Since i.c.v. (as well as systemic) leptin treatment inhibits hepatic *Scd-1* gene expression via a mechanism that cannot be attributed to reduced food intake, leptin regulation of hepatic *Scd-1* gene expression may involve a central site of action (36). Moreover, hepatic insulin resistance induced by HF feeding is blocked by reducing hepatic SCD-1 expression using antisense oligodeoxynucleotides (ASO) (74), and whole-body *Scd-1* deficient mice are protected against DIO and its metabolic sequelae. In addition, introduction of SCD-1 deficiency in lipodystrophic aP2-nSREBP-1c mice corrects hepatic steatosis, although it does not ameliorate diabetes (2), and loss of SCD-1 in *ob/ob* mice reduces body weight and hepatic steatosis but worsens diabetes (62). Thus, although SCD1 regulation likely contributes to effects of leptin on hepatic TG and lipoprotein synthesis, its role in leptin regulation of hepatic glucose metabolism is uncertain.

Another potential mediator of leptin control of glucose metabolism is IGF-binding protein-2 (IGFBP2). Using microarrays, hepatic expression of *Igfbp2* was shown to be up-regulated by leptin, and IGFBP2 plasma levels were also increased following leptin treatment in both *ob/ob* and wild-type mice (80). Moreover, adenoviral-induced overexpression of IGFBP2 normalized blood glucose levels in *ob/ob* mice via improved hepatic insulin sensitivity, accompanied by reduced hepatic expression of the gluconeogenic genes, *G6Pase* and *Pepck*, along with a significant reduction of hepatic steatosis (80). Over-expression of IGFBP2 also reduced blood glucose levels in models of leptin and insulin resistance/deficiency including agouti, DIO and STZ-induced diabetic mice. However, the question of whether IGFBP2 is required to mediate effects of leptin on glucose metabolism remains unanswered, and the mechanism whereby leptin induces *Igfbp2* is also unknown. Additional uncertainty stems from early observations that insulin is a major inhibitory regulator of hepatic *Igfbp2*, such that expression of the latter is markedly increased in animals with insulin-deficient diabetes (67, 68, 194). Since insulin treatment of diabetic rodents lowers both HGP and *Igfbp2* gene expression (26, 138, 194), the notion that IGFBP2 plays a major role in leptin-mediated inhibition of HGP seems counterintuitive. Additional studies are warranted address these questions.

In addition to effects on HGP, at pharmacological doses, central administration of leptin in normal animals increases whole body glucose turnover and glucose uptake in peripheral tissues including skeletal muscle, heart and brown adipose tissue (BAT), but not white adipose tissue (WAT), without changes in plasma insulin or glucose levels (91). The hypothalamus is implicated in these effects since microinjection of leptin directly into the VMH increases insulin-independent glucose uptake in skeletal muscle, heart and BAT (114) via a mechanism involving the sympathetic nervous system (SNS) (77). The observation that these effects of leptin were blocked by the Mc3/4r antagonist, SHU9119 suggests that this effect of leptin is dependent on activation of melanocortin receptors (185). By comparison, leptin injection into the ARC increases glucose uptake only in BAT, while injection into either the LHA (114), DMH or PVN has no effect (185). These findings support a model in which autonomic responses triggered by leptin action in the brain play a key role in changes of peripheral glucose metabolism.

One attractive candidate for mediating the effect of central leptin action on systemic glucose clearance is via autonomic activation of AMPK in peripheral tissues. AMPK is a master sensor of cellular energy expressed in key metabolic tissues including liver, skeletal muscle, adipose tissue and hypothalamus (179), and is activated when fuel availability is reduced, as during exercise, which in turn stimulates fat oxidation while increasing glucose uptake in muscle via an insulin-independent mechanism (154). Moreover, recent evidence suggests

leptin action in the hypothalamus activates AMPK in skeletal muscle via a mechanism involving the sympathetic branch of the autonomic nervous system (115).

However, the mechanism whereby leptin signaling in the CNS communicates to peripheral tissues to stimulate glucose uptake is not well understood. To identify the neural circuits whereby leptin promotes glucose uptake in skeletal muscle, a recent study used LepRb-GFP reporter mice in combination with muscle-specific injection of an mRFP-expressing pseudorabies virus (PRV) (3). They found that while injection of PRV in muscle led to PRV labeling within multiple areas of the hypothalamus and hindbrain, the only area of co-localization between leptin receptor expressing cells and muscle-derived PRV was within the NTS and the retrochiasmatic area (RCh) (3), an area that projects to leptin-sensitive neurons in the intermediolateral (IML) nucleus of the spinal cord (51). While populations may yet exist in other brain areas, these data implicate that the NTS and RCh are key sites whereby leptin action in the brain activates autonomic relays that affect skeletal muscle.

### **G. Extrahypothalamic Neurocircuits Involved in Leptin Regulation of Glucose Metabolism**

One mechanism proposed to explain how leptin reduces food intake is by enhancing the CNS response to gut-derived satiety peptides, such as cholecystokinin (CCK) and glucagon-like-peptide-1 (GLP-1) (196) that are released upon food ingestion and activate vagal afferent fibers that terminate in the NTS (9, 25, 55, 121). Available evidence suggests that leptin signaling in forebrain areas such as the ARC activates a descending projection to the NTS, which in turn, enhances the hindbrain response to input from CCK (122).

Interestingly, a parallel neurocircuit is implicated in the mechanism that links hypothalamic leptin action to the control of hepatic insulin sensitivity, in which leptin-sensitive forebrain neurons regulate the activity of neurons in the NTS and adjacent dorsal motor nucleus of the vagus (DMX). Contained within the DMX are vagal motor neurons that supply the liver and other tissues, and their activity is tonically regulated by input from the NTS. The NTS, in turn, receives input from both afferent vagal fibers and from descending projections from the hypothalamus and other brain areas (Figure 5).

Consistent with this hypothesis, both local administration of insulin and inhibition of fat oxidation in the ARC increase hepatic insulin sensitivity via a mechanism that requires intact hepatic vagal innervation, and also activates hindbrain neurons within both the NTS and DMX (147). Moreover, the beneficial effects of restoring hypothalamic leptin signaling to Koletsky rats on hepatic insulin sensitivity were blocked by selective hepatic vagotomy (66). However, selective hepatic vagotomy failed to affect hepatic glucose metabolism in leptin receptor-deficient Koletsky rats that did not receive leptin gene therapy, and previous studies have demonstrated that selective removal of vagal tone to the liver by itself in genetically normal rats has no effect on insulin-induced suppression of HGP. Similarly, while abundant evidence suggests that vagal afferents play a critical role in the ability of gut-derived satiety signals, such as CCK, to promote the termination of a meal, selective vagotomy by itself fails to affect energy homeostasis (19). Thus, additional studies are required to determine the physiological relevance of this circuit in the control of glucose metabolism.

### **H. Indirect Effects of CNS Leptin on Glucose Metabolism**

While several lines of evidence suggest that leptin action in the brain improves glucose metabolism independent of its effects on energy homeostasis, the effect of leptin to reduce food intake and body weight (and body fat mass) also likely contributes to the overall effects of leptin to improve glucose metabolism, since weight loss as small as 5–10% can substantially improve insulin sensitivity in obese patients (16).

Yet another potential mechanism whereby leptin signaling in the brain can influence insulin action is via effects on lipid metabolism. Infusion of leptin directly into the mediobasal hypothalamus during an euglycemic-hyperinsulinemic clamp suppressed the expression of key *de novo* lipogenic enzymes in WAT and also activated lipolysis and suppressed FFA uptake into this tissue (28). Surprisingly, while the ability of hypothalamic leptin to regulate food intake, glucose metabolism and reproductive function requires intact STAT3 signaling, these effects of leptin on adipose tissue metabolism are STAT3-independent, appearing instead to involve hypothalamic PI3K and SNS outflow (28).

Pertinent to this discussion is the phenotype of mice in which PI3K signaling in leptin-receptor expressing neurons in the CNS is increased by deleting PTEN using cre-loxP technology, alluded to earlier. Selective PTEN ablation in leptin-expressing neurons results in a phenotype characterized by reduced adiposity and increased energy expenditure due, at least in part, to increased fatty acid oxidation in WAT (144), associated with increased sympathetic nerve activity to WAT and leading to a remodeling of WAT to take on characteristics of BAT, including increased mitochondrial content and UCP1 expression (144).

Leptin-mediated activation of the melanocortin pathway may contribute to these potent effects on adipose tissue metabolism. Pharmacological inhibition of melanocortin receptors in the CNS increases WAT lipid uptake, triglyceride synthesis and fat accumulation, whereas activation of neuronal melanocortin receptors stimulates lipid mobilization independent of changes in food intake. Moreover, while reduced melanocortin signaling promotes insulin sensitivity and glucose uptake in WAT, it reduces glucose utilization in BAT and muscle (131), favoring increased TG synthesis and storage in the body.

## V. PERSPECTIVES

### A. Leptin Resistance and Obesity Pathogenesis

While absolute leptin deficiency causes obesity (118, 212), most obese humans and animal models exhibit elevated circulating plasma leptin levels (39). This is not surprising, given that leptin is synthesized and secreted by adipocytes in direct proportion to total body fat mass (39). Yet an abundant literature supports the hypothesis that body fat is regulated by a negative feedback loop described earlier that adjusts food intake and energy expenditure as needed to promote the stability of body fuel stored as fat. These observations raise a fundamental paradox: if body fat is homeostatically regulated, increases of body fat should activate adaptive responses involving increased leptin secretion that protect against weight gain. How, then, do obese individuals maintain elevated weight in the face of elevated leptin levels?

One potential explanation is that under normal conditions, this homeostatic system defends against both weight loss and weight gain, but that common forms of obesity develop due to genetic or acquired defects that impair the response to elevated leptin levels (159). Obesity in both humans and in rodents placed on a HF diet are characterized by hyperleptinemia, and the ability of leptin to suppress food intake and reduce body weight is attenuated (49, 81). Consistent with this observation, the ability of leptin to activate pSTAT3, a marker of leptin signaling (49), is impaired in rodent models of DIO, specifically in the hypothalamic ARC (56, 124). This concept is commonly referred to as “leptin resistance”, and is somewhat analogous to “insulin resistance” whereby elevated insulin levels are required to maintain blood glucose levels in the normal range. Indeed, the same cellular mechanisms that impair insulin signaling in tissues such as liver and muscle of obese individuals may also contribute to the pathogenesis of leptin resistance.



The identification of cellular mechanisms that can explain impaired leptin signaling in DIO is an area of intense current research. One model proposes that during positive energy balance and excess body fat gain, leptin resistance is caused by elevated plasma leptin levels, resulting in chronic overstimulation of the leptin receptor and associated SOCS-3 induction (22, 54, 155). Consistent with this hypothesis, heterozygous SOCS-3 deficient mice display increased leptin sensitivity and are protected against the development of obesity on a HF diet (87). This effect appears to be neurally mediated, as neuron-specific SOCS-3 conditional knockout mice exhibit a similar phenotype (119), suggesting that in neurons, SOCS-3 plays a physiological role to favor positive energy balance and weight gain. Moreover, mutation of Lr<sub>b</sub> Tyr<sub>985</sub> prevents SOCS-3 action on the leptin receptor and thereby results in mice which display increased leptin sensitivity, are lean and are resistant to DIO (24). An important consideration in the interpretation of this work, however, is that pro-inflammatory signaling induced in the hypothalamus during HF feeding (see below) also increases SOCS-3 expression. So while a compelling argument exists in support of a causal role of hypothalamic SOCS-3 in the pathogenesis of common forms of leptin resistance, whether SOCS-3 induction is due to hyperleptinemia, pro-inflammatory cellular responses or some combination of these awaits clarification.

Interestingly, mice expressing a constitutively-active version of STAT3 selectively in POMC neurons causes a hyperphagic, obese phenotype accompanied by central insulin and leptin resistance (58), offering further (albeit indirect) evidence that induction of SOCS-3 in these cells impairs energy homeostasis. Moreover, while up-regulation of SOCS-3 selectively in POMC neurons causes leptin resistance and obesity, somewhat surprisingly, over-expression of SOCS-3 in leptin receptor-positive neurons did not (149). Thus, SOCS-3 may play a cell-type specific role in the regulation of energy balance. Again, it is important to recognize that SOCS-3 is induced not only by pSTAT3, but also by inflammatory signaling through the IKK $\beta$ -NF $\kappa$ B pathway, when interpreting these outcomes.

Similar to SOCS-3, the protein tyrosine phosphatase (PTP)1B inhibits leptin and insulin signaling in cultured cells and *in vivo* via dephosphorylation of Jak2 (48, 93). Consistent with this, HF feeding increases PTP1B expression in the hypothalamus and pharmacological inhibition of PTP1B in the brain improves leptin sensitivity in aged-leptin resistant rats (120). Moreover, neuronal (17) or POMC-specific (7) PTP1B knockout mice are protected from DIO and exhibit increased leptin sensitivity. These data suggest that both SOCS-3 and PTP1B are possible mediators that contribute to reduced LepR<sub>b</sub> signaling in obesity.

A growing literature implicates cellular inflammation in the pathogenesis of obesity-associated metabolic impairment in peripheral tissues (85, 86, 170). Excess body adiposity has been associated with activation of macrophages and associated inflammation in a variety of peripheral tissues including WAT, liver, skeletal muscle and the vasculature and eventually is accompanied by increased circulating plasma levels of pro-inflammatory cytokines (193, 207). Recent evidence suggests that consumption of a HFD also induces inflammation in the hypothalamus, an effect that itself could favor weight gain by impairing the neuronal response to input from leptin and insulin (184). Consistent with this notion, rats fed a HFD for 20-weeks exhibit both leptin resistance and hypothalamic inflammation, as measured by elevated mRNA and protein levels of pro-inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$  and IL-6) resulting from activation of IKK $\beta$  and JNK-1 (45, 148, 211). Beyond these correlative observations, recent work has begun to assess whether hypothalamic inflammation is a cause of DIO, or is simply a consequence.

Consistent with hypothalamic inflammation driving HF-induced obesity, activation of hypothalamic IKK $\beta$ /NF- $\kappa$ B signaling using a gene therapy approach promotes hyperphagia and weight gain and reduces leptin and insulin signaling in mice (211). Conversely,

interventions that reduce hypothalamic inflammation, either by reducing neuronal IKK $\beta$  signaling using a gene therapy approach (211) or by pharmacological administration of a IKK $\beta$  inhibitor, reduce food intake and body weight in DIO animals (148) and maintain central leptin and insulin sensitivity. Thus, hypothalamic inflammation appears to be both necessary and sufficient for HF-induced obesity. This notion does not exclude a pathogenic role for hyperinsulinemia, since both inflammatory signaling and leptin share in common the ability to induce SOCS-3.

Further evidence of a role for SOCS-3 in the pathogenesis of DIO stems from mice with cell-type specific deletion of IKK $\beta$  in AgRP neurons, which are protected against DIO and exhibit reduced expression of SOCS-3 in the mediobasal hypothalamus (211). Moreover, increasing SOCS-3 expression in the mediobasal hypothalamus using a lentiviral approach blocked the protection against DIO conferred by AgRP-neuron-specific IKK $\beta$  knockout (211). Although the extent to which leptin resistance induced by hypothalamic inflammation depends upon SOCS-3 remains to be established, these data suggest that both hyperleptinemia and inflammation may contribute to central leptin resistance via an overlapping mechanism involving SOCS-3.

Yet another mechanism that may contribute to hypothalamic inflammation and central leptin and insulin resistance is endoplasmic reticulum (ER) stress. Recent studies demonstrate that ER stress and the unfolded protein response (UPR) are activated in the hypothalamus of obese mice and that this effect is sufficient to inhibit leptin signaling (140). In support of the hypothesis that ER stress is involved in pathways that mediate nutritional activation of hypothalamic IKK $\beta$ /NF- $\kappa$ B, central administration of an ER stress inhibitor suppressed hypothalamic NF- $\kappa$ B levels in HF-fed rats and lowered both food intake and body weight in DIO animals (211). Conversely, induction of ER stress in the brain of mice and rats increases food intake and body weight and blocks the anorexic effects of insulin and leptin (140, 199). In addition, neuronal deletion of X-box binding protein-1 (XBP-1) in mice increased food intake and body adiposity, and is accompanied by hypothalamic leptin resistance (140).

To compare the contribution of hyperleptinemia versus a HFD itself to the development of leptin resistance a recent study treated *ob/ob* mice with systemic leptin continuously at a dose designed to “clamp” plasma levels to that of wild-type controls fed standard chow (94). Following the switch to a HFD, wild-type and leptin-infused *ob/ob* mice displayed similar weight gain despite markedly higher plasma leptin levels in the former group (94). Moreover, wild-type mice exposed to a HFD were resistant to the ability of leptin to reduce food intake and body weight and to induce hypothalamic pSTAT3 relative to leptin-infused *ob/ob* mice fed a HFD. The authors' concluded from these studies that hyperleptinemia is required for the development of leptin resistance (94). However, the important question of whether hyperleptinemia is sufficient to induce leptin resistance was not examined in this study (since *ob/ob* mice did not receive leptin at doses that induce hyperleptinemia) and perhaps more importantly, the study design presumes that wild-type and leptin-infused *ob/ob* mice are fundamentally indistinguishable where leptin sensitivity and energy homeostasis are concerned, despite clear evidence that *ob/ob* mice are more sensitive to leptin than normal mice (30, 76). Additional studies are therefore needed to determine the extent to which elevated plasma leptin levels contribute to leptin resistance and associated weight gain during HF feeding.

One conclusion stemming from this area of science is that viable mechanisms have finally emerged with the potential to link altered hypothalamic function to sustained weight gain in models of DIO (127). A second inference is that no single mechanism is likely predominate in the pathogenesis of obesity-associated leptin resistance and, like insulin resistance in

peripheral tissues, it seems probable that numerous mechanisms contribute in ways that are challenging to disarticulate.

## B. Physiological Relevance

The regulation of blood glucose levels is a complex and highly integrated process involving the brain and peripheral tissues. In this review, we have highlighted evidence that the adiposity signal leptin plays an important role in the control not only of energy homeostasis, but of glucose homeostasis as well, and that the CNS is implicated in both leptin effects. Yet most approaches used to investigate the role of leptin in glucose homeostasis employ either genetic models (e.g. *ob/ob* mice, *db/db* mice or *fa<sup>k</sup>/fa<sup>k</sup>* rats) or pharmacological doses of leptin in normal animals. While this research has generated a wealth of information and has advanced our understanding of how leptin acts in the CNS and how CNS leptin action is communicated to the periphery, whether these leptin effects play a physiological role in glucose homeostasis on a minute-to-minute, hour-to-hour and day-to-day basis in genetically normal humans and animal models, and whether such effects are neurally-mediated, are key questions that remain incompletely answered.

Other technological considerations warrant comment. To examine the physiological mechanisms whereby insulin inhibits HGP *in vivo*, many experiments employed the pancreatic clamp, a technique that employs the infusion of somatostatin to block endogenous insulin and glucagon secretion, along with i.v. infusion of insulin and glucagon to maintain basal levels. One limitation of this approach relates to the fact that endogenous insulin is secreted into the hepatic portal vein, such that the liver is exposed to insulin levels higher than in the systemic circulation. Since most studies do not account for this difference (with the consequence that systemic and hepatic portal vein levels of insulin are matched), the question of whether neural sensing of insulin (or leptin) participate in the control of HGP has been challenged based on evidence that under true physiological conditions, insulin action on the liver is mediated primarily via a direct effect (32). While most investigators can agree that the brain senses changes of circulating insulin levels, whether insulin action in the brain plays a physiological role in the day-to-day regulation of glucose production by the liver or other tissues remains to be firmly established, and the same may be said about the role of leptin in glucose metabolism.

## VI. CONCLUDING REMARKS

In conclusion, a large and growing literature supports the hypothesis that input to the CNS from afferent signals such as leptin is integrated with that from a variety of other hormonal and nutrient-related signals to co-ordinate efferent responses that control food intake, energy expenditure, hepatic insulin sensitivity and glucose uptake in the service of energy and glucose homeostasis. Evidence of substantial overlap in the CNS circuits that regulate energy balance and those that regulate glucose metabolism raises the possibility that reduced secretion, sensing, or responsiveness to these afferent signals promotes both weight gain and insulin resistance. Therapies that target the CNS and restore sensitivity of neuronal circuits to key afferent signals have the potential to improve currently available treatments for obesity and diabetes.

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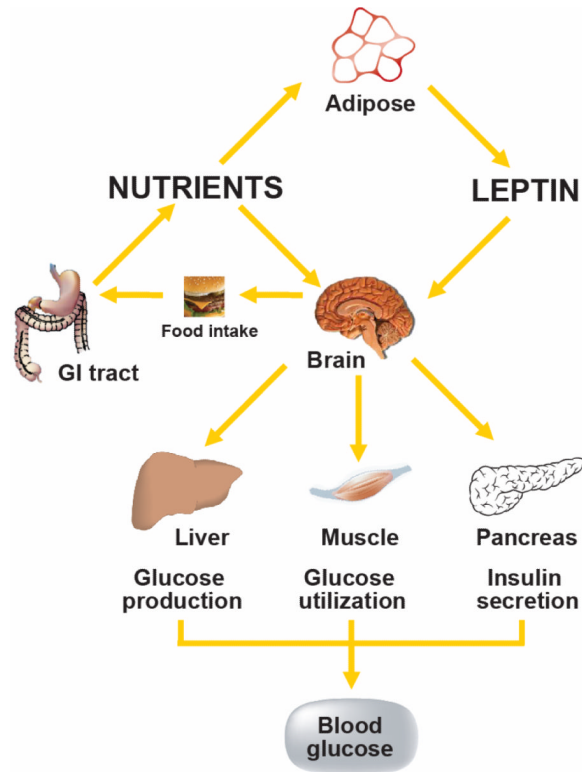
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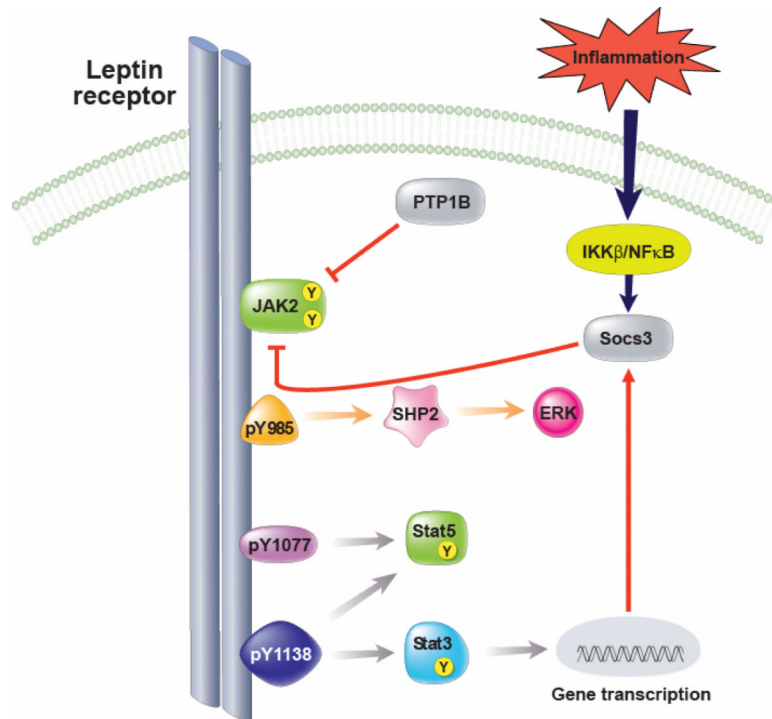
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**FIGURE 1. Model of CNS regulation of glucose homeostasis**

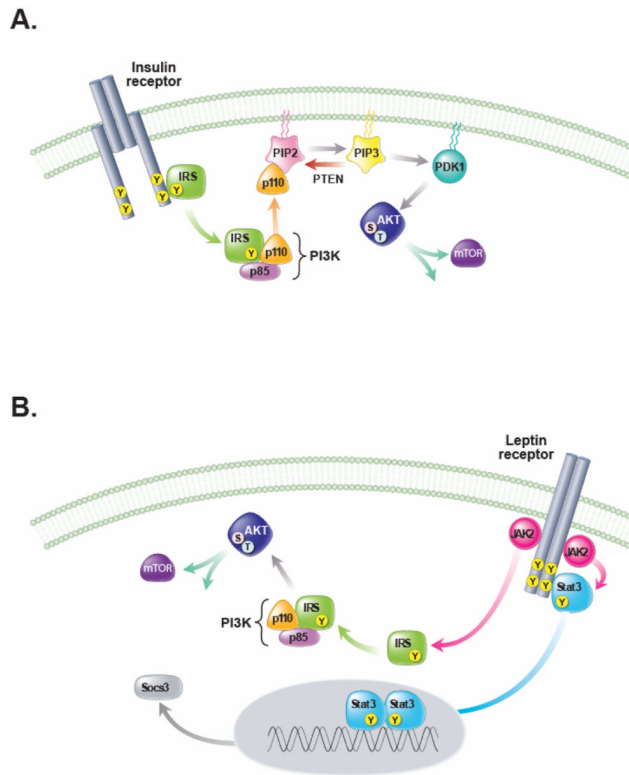
The CNS integrates signals from both long-term energy stores (e.g. leptin) and short-term availability (nutrients; e.g. free fatty acids) and orchestrates responses that coordinate hepatic glucose production, peripheral glucose uptake and insulin secretion to maintain glucose homeostasis.





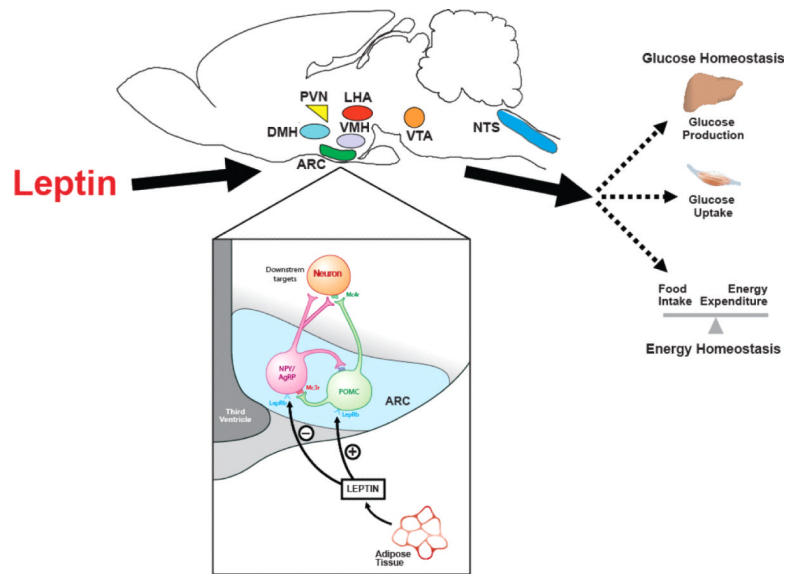
### FIGURE 2. Leptin receptor signaling

Leptin binding to LepRb mediates activation of the tyrosine kinase Janus kinase-2 (Jak2), resulting in phosphorylation of three tyrosine residues (Tyr<sub>985</sub>, Tyr<sub>1077</sub>, Tyr<sub>1138</sub>). Phosphorylation of Tyr<sub>985</sub> recruits SHP-2, leading to the activation of the ERK pathway, and also binds to suppressor of cytokine signaling-3 (SOCS-3), a negative regulator of LepRb signaling. Phosphorylation of Tyr<sub>1077</sub> mediates signal transduction and activation of transcription factor-5 (STAT5). Phosphorylation of Tyr<sub>1138</sub> recruits and activates STAT3 and STAT5. Activation of STAT3 leads to increased SOCS-3 expression which acts as a negative feedback inhibitor of leptin signaling, in part, by binding to Tyr<sub>985</sub> of LepRb. In addition, hypothalamic inflammation is also induced during high-fat feeding and increases SOCS-3 expression. Similar to SOCS-3, protein tyrosine phosphatase (PTP)1B may also inhibit LepRb signaling via dephosphorylation of Jak2.



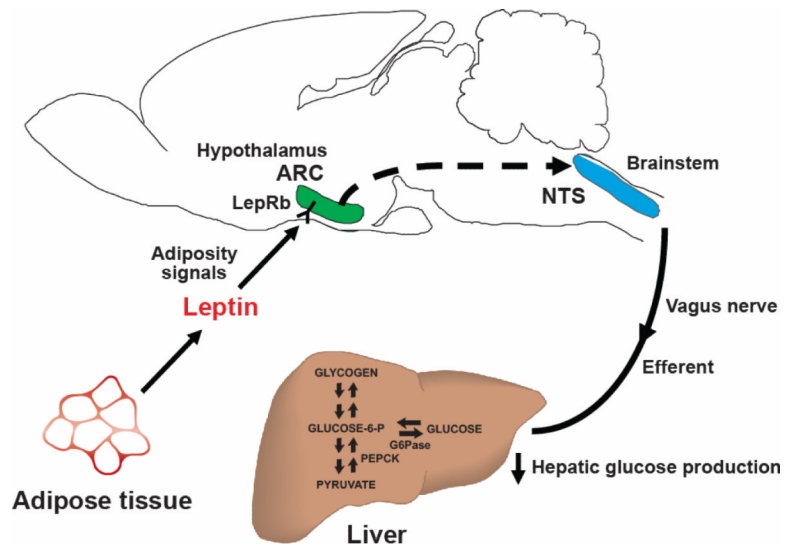
**FIGURE 3. Leptin and insulin signal transduction pathways in the CNS**

**A.** Activation of the insulin receptor induces tyrosine phosphorylation of insulin receptor substrate (IRS) proteins which in turn activate phosphatidylinositol-3-kinase (PI3K), an enzyme that phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>) to phosphatidylinositol-3,4,5-trisphosphate (PIP<sub>3</sub>) and activates several downstream molecules including 3-phosphoinositide-dependent protein kinase 1 (PDK1), protein kinase B (PKB, also referred to as Akt) and mammalian target of rapamycin (mTOR). **B.** Leptin binding to the extracellular domain of the LepRb activates Janus kinase-2 (JAK2), leading to binding and tyrosine phosphorylation of STAT3, which dimerizes, translocates to the nucleus and activates transcription of target genes SOCS-3, which can attenuate both insulin and leptin receptor signaling. Like insulin, leptin signaling can also activate PI3K, and downstream targets, presumably via tyrosine phosphorylation of IRS proteins by Jak2. Although leptin and insulin both have the potential to activate IRS-PI3K signaling in neurons and other cell types, important differences in the response to these two hormones exist. These likely arise from divergent roles for PI3K signaling within a given cell type due to differences in the intracellular microdomain in which PI3K signaling is activated, or from distinct cell subpopulations that respond to either leptin or insulin, but not both (e.g. POMC neurons). PTEN, phosphatase and tensin homologue.



**FIGURE 4. CNS neurocircuits regulating energy and glucose homeostasis**

A number of hypothalamic and extrahypothalamic sites have been implicated in the action of leptin to regulate energy and glucose homeostasis. The hypothalamic ARC contains neuropeptide Y and agouti-related peptide (NPY/AgRP) neurons that stimulate food intake and are inhibited by leptin, and pro-opiomelanocortin (POMC) neurons that reduce food intake and are stimulated by leptin. NPY/AgRP neurons also inhibit POMC neurons via synaptic release of the neurotransmitter  $\gamma$ -aminobutyric acid (GABA). ARC, arcuate nucleus; LepRb, leptin receptor; Mc3r/Mc4r, melanocortin-3/4 receptor; VMH, ventromedial hypothalamus; LHA, lateral hypothalamic area; PVN, paraventricular nucleus; DMH, dorsomedial hypothalamus; VTA, ventral tegmental area; NTS, nucleus of the solitary tract.



**FIGURE 5. Model of the hypothalamic regulation of hepatic glucose production**

Leptin-sensing neurons in the hypothalamic ARC receive input regarding energy stores and in response to this input, pathways that increase hepatic vagal tone to the liver are activated, increasing hepatic insulin sensitivity. ARC, arcuate nucleus; LepRb, leptin receptor; NTS, nucleus of the solitary tract; G6Pase, glucose-6-phosphatase; PEPCK, phosphoenolpyruvate kinase.