Leptin and Insulin Action in the Central Nervous System

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Body adiposity is known to be carefully regulated and to remain relatively stable for long periods of time in most mammalian species. This review summarizes old and recent data implicating insulin and leptin as key circulating signals to the central nervous system, particularly the ventral hypothalamus, in communicating the size and the distribution of body fat stores. This input ultimately alters food intake and energy expenditure to maintain constancy of the adipose depot. The key primary neurons in the arcuate nucleus containing NPY/AgRP and POMC/CART appear be critical constituents of the CNS regulating system, and are shown to contribute to anabolic and catabolic signaling systems to complete the feedback loop. New data to indicate shared intracellular signaling from leptin and insulin is provided. The satiety system for meals, consisting of neural afferents to the hindbrain from the gastrointestinal tract, is described and its effectiveness is shown to vary with the strength of the insulin and leptin signals. This provides an efferent mechanism that plays a key role in a complex feedback system that allows intermittent meals to vary from day to day, but provides appropriate long-term adjustment to need. Recently described contributions of this system to obesity are described and potential therapeutic implications are discussed.

Key Words: adiposity, insulin, leptin, ventral hypothalamus, anabolic, catabolic signaling, satiety system

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Introduction

The impressive stability of body adiposity over long periods of time, despite often marked variation in daily food intake and energy expenditure, led Kennedy to propose that a signal generated in proportion to body fat stores acts in the brain to promote energy homeostasis.[1] Initially, a variety of circulating nutrients were proposed to function as "adiposity signals" to the brain, but studies performed over many years failed to support this possibility.[2] Twenty years later, Coleman surgically coupled (using parabiosis) normal mice with mice with genetic obesity (*ob/ob* or *db/db*) so as to create a cross-circulation between two animals: one normal and one obese. His demonstration of profound changes in food intake and body weight of the parabiotic partner in this coupling led to the conclusion that the *ob* locus is necessary for the production of a humoral satiety factor, whereas the *db* locus encoded a molecule required for the response to this factor.[3,4]

Ten years later, Woods and Porte demonstrated an increase in cerebrospinal fluid (CSF) insulin levels during peripheral insulin infusion,[5] thereby establishing that insulin is taken up into the central nervous system (CNS) from the bloodstream. Recognizing that serum insulin circulates at concentrations proportionate to body adiposity,[6] they tested the hypothesis that insulin provides feedback to the CNS to participate in food intake and body weight regulation. To do so, they infused insulin over a period of weeks into the lateral cerebral ventricle of freely feeding baboons. Their observation of a dose-dependent decrease of daily food intake and body weight provided initial, compelling support for the notion that insulin is a signal from the periphery to the CNS that regulates body adiposity.[7] On the other hand, because insulin levels are extremely elevated in *ob/ob* mice, the missing circulating factor in these animals could not be insulin.

Some 15 years later, the *ob* gene was cloned[8] and shown to encode a hormone that is secreted from adipocytes in direct proportion to the amount of stored fat via mechanisms that are sensitive to both the ongoing metabolic activity of fat tissue and total body fat content. Shortly thereafter, the recombinant molecule, termed "leptin," was shown to significantly inhibit food intake

and body adiposity when given either peripherally or centrally.[9] Because it was far more potent on a molar basis when given centrally than peripherally, it was concluded that leptin is, similar to insulin, a peripheral signal to the CNS that participates in the long-term regulation of body weight.

In the intervening years, insulin receptors were identified in brain and shown to be concentrated in the hypothalamus[10] and to have binding and signaling properties quite similar to the peripheral insulin receptor.[11,12] Circulating insulin was thought to have access to brain insulin receptors in part because of a reduced blood-brain barrier in the median eminence and circumventricular organs,[13] and in part because brain capillary endothelial cells can facilitate transcapillary insulin transport.[14] Both studies of endothelial cells in vitro and analysis of the pattern of uptake of insulin from blood to CSF in vivo[15] were consistent with a receptor-mediated transport system as a means for regulated insulin delivery to critical CNS regions involved in energy homeostasis.

Neuropeptide Y (NPY) was the first neuropeptide-signaling molecule to be identified as a potential mediator of these regulatory effects of insulin. Expressed in the ventral hypothalamus, particularly the arcuate nucleus, NPY expression was shown to be selectively increased in insulin-deficient, streptozocin-induced diabetic rats and that this effect is reversed by insulin replacement therapy.[16,17] Because infusion of NPY into cerebral ventricles is a powerful stimulus to food intake, it was concluded that insulin deficiency in the diabetic syndrome led to an increase of NPY biosynthesis and release that was responsible, at least in part, for the hyperphagia associated with poorly controlled diabetes mellitus.

Early discoveries in the emerging field of leptin biology followed a parallel path. Cloning of the leptin receptor revealed it to be present in the CNS, with the "signaling" long form of the receptor concentrated in ventral hypothalamic areas including the arcuate nucleus,[18] the same brain area implicated as a key regulatory sensor for the effects of insulin on energy homeostasis. Subsequently, uptake of leptin, this much larger 16-kD molecule, into the CNS was hypothesized to involve a similar receptor-mediated endothelial cell transcytotic mechanism.[19] Several studies support this hypothesis and suggest that the rate is reduced in obesity.[20,21]

Body Weight Regulation

As described in rodent models of insulin deficiency, increased hypothalamic expression of NPY mRNA and peptide was shown to be present in leptin-deficient *ob/ob* mice and to be reversed by the administration of leptin either systemically or centrally.[22,23] Thus, both leptin and insulin were identified as putative candidate adiposity signals. Both hormones circulate at levels proportionate to body fat content, and enter the CNS in proportion to their plasma level. Insulin receptors and leptin receptors are expressed by brain neurons involved in energy homeostasis and administration of either peptide directly into the brain reduces food intake and NPY gene expression, whereas deficiency of either hormone produces hyperphagia.

Whereas insulin and leptin are the only molecules known to fulfill these criteria, the information provided to the CNS by these two signals appears to differ in some respects. Insulin levels are determined largely by peripheral insulin sensitivity, which is related not only to total body fat stores,[24] but is particularly sensitive to fat distribution. Thus, central or intra-abdominal fat appears to be a key determinant of whole-body insulin sensitivity and plasma insulin levels.[25] In addition, insulin secretion (but not leptin secretion) is rapidly responsive to food ingestion and changes dramatically with every meal throughout a 24-hour period.[26] On the other hand, whereas leptin levels are sensitive to total body fat mass, they are relatively unresponsive to meal ingestion; leptin levels are quite sensitive to caloric balance and will drop drastically when total caloric intake is markedly reduced or increase when total caloric intake is excessively elevated.[27,28] In addition, leptin is released from fat stores by mechanisms that appear to involve glucose flux in adipocytes.[29,30] Because adipose tissue is highly insulin sensitive, leptin secretion is itself directly under the influence of insulin action in fat stores. There is also a circadian rhythm to leptin secretion with highest levels in humans observed after midnight, well before the morning meal.[31] Thus, both the insulin and leptin signaling systems are sensitive to the state of energy balance and body fat stores and are redundant in some ways, but complementary in others.

Central Nervous System Mechanisms

In recent years, a major focus has been placed on the identification of neuronal systems targeted by these two molecules, and the intracellular signaling systems upon which they act. What has become apparent is that their actions in the CNS are again partially complimentary and partially redundant. An example of their redundancy is the effect of either molecule to reduce food intake when given to rats with uncontrolled, insulin-deficient diabetes[32,33] that are both insulin- and leptin-deficient. Leptin appears to be more effective than insulin; systemic leptin replacement completely prevents diabetic hyperphagia, whereas centrally administered insulin only partially reverses it. Furthermore, insulin does not reduce food intake in *fa/fa* rats with defective leptin.[34] Thus, intact leptin signaling appears to be required for insulin to exert

its central effects. But CNS insulin action may also be necessary for intact neuronal responsiveness to leptin. This conclusion stems from evidence that CNS insulin resistance induced by genetic deletion of either the brain insulin receptor[35] or the insulin receptor–signaling molecule, IRS-2,[36] causes pathologic expansion of fat stores despite elevated leptin levels; this also suggests the presence of CNS leptin resistance.

In attempting to unravel this complex interaction, several distinct hypothalamic neuropeptide-containing pathways have emerged as being both critical participants in body weight regulation and sensitive to insulin and/or leptin signaling. Some of these molecules (like NPY) are termed "anabolic" because they increase daily food intake and decrease energy expenditure when administered centrally. Conversely, others are catabolic, in that they reduce food intake and increase energy expenditure. The most prominent member of the latter group, and one of the first to be characterized is α-melanocyte-stimulating hormone (α-MSH), a key member of the melanocortin family. α-MSH is a neuropeptide cleaved from the pro-opiomelanocortin (POMC) precursor molecule and is a ligand for several members of the family of melanocortin receptors. Of special interest among these are the melanocortin 3 receptor (MC3R) and melanocortin 4 receptor (MC4R) that are expressed primarily in the brain. The report that mice with genetic knockout of the MC4R gene are obese,[37] whereas a synthetic agonist of these receptors suppresses food intake,[38] indicates that signaling by MC4R tonically limits food intake and body fat mass. Genetic deletion of the MC3R also leads to excessive body fat deposition, but the effect is mild and does not involve increased food intake.[39] POMC neurons are present in the arcuate nucleus, and similar to NPY neurons in this area,[40] have been shown to express leptin receptors.[41] Regulation of these POMC neurons by leptin was suggested by reduced levels of POMC mRNA found in the arcuate nucleus of *ob/ob* mice that are restored to normal values by leptin administration.[42] This regulation of POMC gene expression by leptin is precisely opposite to that of NPY mRNA in arcuate nucleus—NPY mRNA is increased in *ob/ob* mice and is reduced by leptin.[22]

Leptin administration to normal rats is also associated with reduced food intake accompanied by an increase of POMC mRNA in the arcuate nucleus.[43] Similarly, involuntary overfeeding via intragastric nutrient infusion elicits profound anorexia in rats that is accompanied by a threefold increase of POMC mRNA.[44] Both responses are reversed by central administration of SHU9119, an MC4R antagonist, even when given at a low doses that do not independently affect food intake. Such observations confirm the importance of the melanocortin system in the central effects of leptin.[44,45]

Whereas direct evidence for regulation of this system by insulin awaits further study, POMC mRNA is reduced by 80% in rats with untreated diabetes, and this decrease can be attenuated by systemic insulin treatment with a dose that partly reversed the hyperglycemia.[33] Because animals with uncontrolled diabetes are also leptin deficient, and because this leptin deficiency is partly reversed by insulin treatment,[32] the relative contributions of insulin and leptin to hypothalamic neuropeptide expression and feeding responses in this model are presently unknown. However, the finding that either hormone can partly or completely reverse the hyperphagia of diabetes again indicates the considerable redundancy that characterizes the CNS effects of the two hormones. Insulin-deficient diabetes is therefore associated with both an increase in hypothalamic NPY and a decrease in hypothalamic POMC biosynthesis, and both responses are reversed by insulin treatment. These findings implicate the arcuate nucleus as a major site for transducing afferent input from circulating leptin and insulin into neuronal responses that control daily food intake and body weight. The complexity of these systems is further amplified by the finding of two additional neuropeptides synthesized by the same subsets of arcuate nucleus neurons, both of which have powerful effects on food intake. Agouti-related peptide (AgRP) is an endogenous antagonist of both melanocortin 3 receptors (MC3R) and melanocortin 4 receptors (MC4R),[46] and is expressed exclusively in arcuate nucleus NPY-containing neurons.[47] Because inhibition of MC4R also increases food intake and body weight, the simultaneous release of NPY and AgRP is proposed to amplify the ability of insulin and leptin to influence food intake via regulation of this neuron.

Similarly, arcuate nucleus POMC neurons co-express cocaine-amphetamine-regulated transcript (CART),[48] a recently described neuropeptide that inhibits both normal and starvation-induced feeding when injected intracerebroventricularly (ICV) in rats. In addition, ICV CART can completely block the feeding response induced by ICV NPY and an anti-serum raised against CART was shown to increase feeding following ICV injection in normal rats.[49] Like POMC mRNA, CART mRNA expression is markedly reduced in the arcuate nucleus of mice with obesity owing either to reduced leptin levels or to reduced leptin signaling.[50] Because this neuropeptide is expressed widely throughout the brain, (unlike POMC) its exact site of action appears to be complex. Nevertheless, its metabolic effects and co-expression in POMC neurons identifies a second set of dually expressing neuropeptide neurons that are regulated by leptin and that amplify one another's effects to decrease daily food intake. Leptin administration induces *fos* expression (a marker of neuronal activation) in these POMC/CART neurons and in other CART-expressing neu-

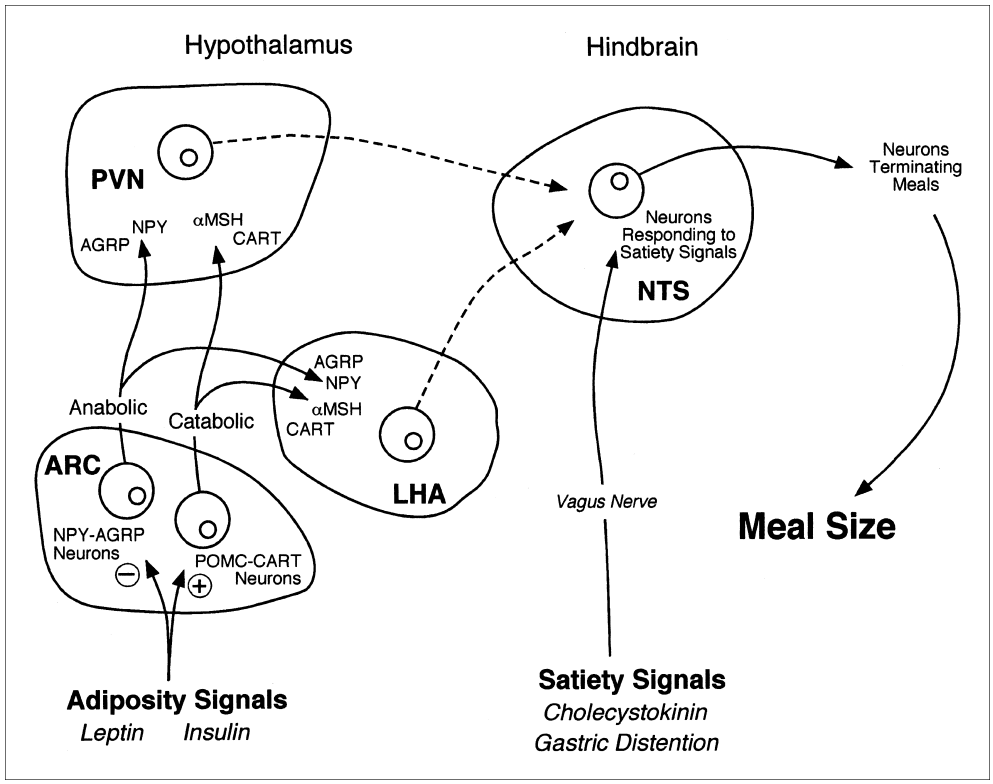

Figure 1. Hypothesized model of central nervous system (CNS) pathways for integration of adiposity signals and satiety signals. Insulin and leptin are adiposity signals that act in the arcuate nucleus (ARC) of hypothalamus to inhibit expression of neuropeptide Y/agouti-related peptide (NPY/AGRP) neurons that promote an anabolic state, (increased food intake, decreased energy expenditure) and stimulate expression of pro-opiomelanocortin/cocaine and amphetamine–regulated transcript (POMC/CART) neurons that promote catabolic effects (anorexia, increased energy expenditure). Release of neuropeptides secreted by these neurons into the paraventicular nucleus (PVN) and the lateral hypothalamic area (LHA) is proposed to regulate downstream neuronal pathways (shown in dashed lines). Such "second-order" neurons mediate effects of insulin and leptin by regulating the response of neurons in the nucleus of the solitary tract (NTS) in the hindbrain to satiety signals (such as cholecystokinin and gastric distention), generated by ingestion of a meal. Such satiety signals arrive at the NTS primarily via the vagus. Descending catabolic and anabolic pathways are proposed to alter the response of NTS neurons that regulate the termination of meals. Catabolic pathways increase, and anabolic pathways decrease, the effect of short term satiety signals, thereby causing chronic adjustments in meal size that have the long term effect of altering food intake and body weight. The specific neurons comprising these pathways between hypothalamus and NTS in this model have not been identified. αMSH = α-melanocyte-stimulating hormone.

rons in the hypothalamus.[48] The CNS effects of leptin are therefore complex and involve the same neuropeptides in neurons additional to those in the arcuate nucleus (e.g., NPY/AgRP and POMC/CART neurons).

Information is also emerging regarding the site of action of arcuate nucleus neurons involved in energy homeostasis. Because lesions of the paraventricular nucleus (PVN) are known to cause obesity,[51] whereas lesions of the lateral hypothalamic area (LHA) produce a syndrome of transient anorexia followed by defense of a lower body weight,[52] coordination of these two nuclei by input from insulin- and leptin-sensing arcuate nucleus neurons provides a signal amplification step for control of energy balance. In response to weight loss, for example, appropriate activation of neurons in LHA neurons, combined with reduced output from anorexigenic cell groups in the PVN, could increase food intake and reduce energy expenditure and thereby promote fat storage. Conversely, increased signaling via these PVN neurons could reduce daily food intake and increase energy expenditure to promote depletion of fat stores, if required to promote homeostasis. In support of this notion are the abundant projections from both NPY/AgRP and POMC/CART neurons to both PVN and LHA.[53–55] However, signaling from the PVN and LHA may be complex in that neurons that originate in these nuclei also terminate in the arcuate nucleus. In addition to projections to brain areas outside of the hypothalamus, intrinsic (or short-loop) neuronal feedback is therefore likely to participate in the coordination of hypothalamic neuronal signals involved in energy homeostasis. (See figure 1).

Among several weight-regulating neuronal signaling molecules in the PVN are corticotrophin-releasing hormone (CRH), oxytocin, and thyrotrophin-stimulating hormone (TSH); conversely, melanocyte-concentrating hormone (MCH) and orexins A and B are found in the

LHA.[56–58] Each of the PVN peptides (CRH, oxytocin, and TRH) can reduce food intake and trigger sympathetic nervous system stimulation and various neuroendocrine responses[59–61] (e.g., stimulation of the hypothalamic-pituitary-adrenal axis by CRH, and of the thyroid hormone axis by TRH) that also influence energy balance. The importance of neurons containing MCH in the lateral hypothalamic area/perifornical area (LHA/PFA) is emphasized by the reduced food intake and excessive leanness that characterizes MCH knockout mice.[62] Data obtained by targeted deletion of the orexin A– and B–producing gene (both orexins are encoded by a single gene) suggests that although these peptides can increase food intake, the predominant effect of loss of orexin function is a narcolepsy syndrome, without a clear body weight phenotype.[63] Thus, whereas food intake and body weight regulation are important functions of these neuropeptides, they are involved in other complex behaviors and a wide array of metabolic processes.

Energy expenditure is an important contributor to body weight regulation that is also sensitive to leptin. For example, animals with obesity owing to mutations of either leptin (*ob/ob*) or the leptin receptor (*db/db, fa/fa*) have markedly reduced oxygen consumption at rest, and thus will gain weight and adipose mass even when pair-fed to the intake of controls.[64] Furthermore, leptin deficiency is suggested to play a critical role in the neuroendocrine, behavioral, and autonomic response to starvation.[65] The effects of fasting on thyroid function, reproductive hormones, the HPA axis, and sympathetic outflow to adipose tissue (particularly brown fat in rodents), are therefore attenuated by leptin administration. Furthermore, abnormal regulation of neuroendocrine and autonomic function has been demonstrated in rodents with either leptin deficiency or inadequate leptin signaling, including the *ob/ob* and *db/db* mouse and the *fa/fa* rat.[64] Thus, a component of the weight gain in these animals can be attributed to increased energy efficiency, which enhances the effect of hyperphagia to promote caloric storage.

Neuronal melanocortin signaling is a potentially important intermediary in these leptin effects because pharmacologic blockade of the MC4R reduces body temperature and spontaneous locomotor activity, while increasing food intake and peripheral leptin levels.[66] Leptin's effects on the thyroid axis also involve hypothalamic signaling via MC4R.[67,68]

Satiety and the Control of Single-meal Intake

The previous discussion has focused on a feedback system between the peripheral energy stores in fat that is coupled through complimentary anabolic and catabolic neural signaling systems with first-order neurons in the arcuate nucleus that respond to insulin and leptin (Figure 1). Both hormones suppress anabolic-signaling pathways that promote weight gain, while stimulating catabolic neuronal systems that cause anorexia and increase energy expenditure. In response to a sustained energy deficit, therefore, fat stores become depleted, and the consequent fall in insulin and leptin levels generates a signal transduced by the CNS into increased food intake and decreased energy expenditure. Regardless of the level of these signals, however, intermittent meals continue to be taken, the size and frequency of which are regulated by a set of neural and hormonal satiety signals generated primarily within the gastrointestinal tract. These signals collectively provide information to the brain that limit the size of a single meal by accelerating its termination. Some of these signals arise from mechanical inputs, such as distention in the gastrointestinal tract, and many are transmitted to the hindbrain via afferent fibers of the Vagus nerve. Other, perhaps more important signals are gastrointestinal peptides, the secretion of which is stimulated by the ingestion of individual nutrients. Cholecystokinin (CCK) is one such hormonal component of this system and was the first gastrointestinal satiety peptide to be identified, originally from studies by Gibbs and Smith more than 25 years ago.[69]

Transmitted to hindbrain areas via the Vagus nerve, input from CCK and other individual meal factors comprise a potentially important site at which the control of single-meal intake is adjusted according to long-term energy requirements. Thus, as CNS insulin or leptin signaling increases, sensitivity to CCK also increases,[70–72] thereby decreasing the size of single meals. Conversely, deficient neuronal input by insulin or leptin is proposed to blunt responsiveness to satiety signals and hence, to increase meal size. In this way, meal size can vary considerably from meal to meal depending upon behavioral and a wide variety of other factors. Over time, however, the total amount of food eaten per day will be responsive to the strength of the insulin and leptin signals (via changes in responsiveness to satiety signals), which in turn reflect the size of adipose energy stores. This model is proposed to explain how body adiposity is maintained remarkably constant over long periods of time despite marked variation in meal patterns, diet composition, and meal size from meal to meal and one day to the next.

The nucleus of the solitary tract (NTS) in the posterior hindbrain is a major integration area for afferent vagal input from the gastrointestinal tract,[73] with projections both from and to the ventral hypothalamus. In addition, neurons in the vicinity of the NTS express MC4 and leptin receptors, and POMC neurons are present as well, suggesting that the hindbrain may process information involved in energy homeostasis directly. The activity of these receptors is demonstrated by (1) the local administration of MC4R agonists or antagonists into the

fourth ventricle, an intervention that elicits feeding responses indistinguishable from those induced by injecting these compounds into the lateral ventricle,[74] and (2) by infusing leptin into the fourth ventricle and demonstrating inhibition of gastric emptying.[75] Thus the feedback loop for single-meal satiety can be modified in complex ways to promote the integration of single meals with the long-term loop for body weight regulation at several levels in the CNS (Figure 1).

Intracellular Signals

The intracellular signaling mechanisms that participate in insulin and leptin action in the CNS have also been under study for some years and many of the details are well described. However, new information indicates that whereas the previously recognized signaling systems suggested completely independent mechanisms signaling downstream of insulin and leptin receptors, this may not be the case. Rather, cross-talk between these two receptor systems now appears to be likely, with complex interactions almost certainly present in most cells where the two receptors are expressed. The insulin receptor consists of an extracellular alpha subunit involved in ligand binding, and an intracellular beta subunit that transduces the insulin signal as a tyrosine kinase. The classical insulin receptor substrates, IRS-1 and IRS-2, have both been identified in neurons,[36,76] but initial studies with IRS-1 knockout animals did not demonstrate an obvious food intake or body weight altering phenotype.[77] Thus, whereas neuronal insulin receptors are capable of phosphorylating IRS-1, either its degree of phosphorylation or the relative amount of IRS-1 is low, and therefore IRS-1 may not play a major role in neuronal insulin signaling in adult animals. However, the clear importance of the brain insulin signaling system was recently demonstrated when the neuronal insulin receptor was selectively knocked out.[35] The phenotype of these animals includes a modest and variable increase in food intake, a consistent increase in adipose tissue fat storage, an increase in peripheral insulin and leptin concentrations, and a defective gonadotrophin secretion leading to limited reproductive capacity. Almost simultaneously, mice with knockout of IRS-2 were reported. While producing a complex phenotype owing to non-neuronal effects of the knockout in the periphery, the phenotype of IRS2 knockout mice is also associated with an increase in food intake, increased fat stores, and infertility with impaired gonadotrophin hormone secretion. Combined with evidence that IRS-2 mRNA is concentrated in the arcuate nucleus, these observations suggest that the neuronal insulin receptor is physiologically coupled to IRS-2 rather than to IRS-1.[36]

Another signaling molecule that could contribute to the neuronal effects of insulin is the protein encoded by the *tub* gene, which is tyrosine phosphorylated by the insulin receptor.[78] Because heterozygous loss of function of the *tub* gene is associated with obesity,[79] the body weight and reproductive defects resulting from loss of neuronal insulin signaling could be related to defective signaling by this protein (designated "tubby"). The function of tubby was recently clarified by an elegant series of experiments revealing it to be an intracellular signaling molecule anchored to the plasma membrane by IP (4,5) P_2. In response to activation of Gαq-coupled receptors, IP_2 is hydrolyzed through activation of phospholipase-C. Tubby is then translocated to the cell nucleus, where it appears to act as a transcription factor.[80] Although the identity of specific G–protein coupled receptors that signal via tubby remain to be determined, MCH and at least one serotonin receptor subtype activate Gαq (specifically the 5-HT2C receptor[81,82]). Tubby phosphorylation by insulin has not been shown in vivo, but because both the melanocyte-concentrating hormone receptor (MCHR) and the 5-hydroxytryptamine 2C receptors (5-HT2CRs) are implicated in food intake and body weight regulation,[62,82] an interaction among the MCH, serotonin, and insulin-signaling systems involving tubby is possible.

Another potential site of intracellular interaction involves a molecule, phosphatidylinositol 3 kinase (PI-3 kinase), identified as a downstream effector of IRS-1 and IRS-2 signaling. Whereas leptin receptor activation also leads to tyrosine phosphorylation of intracellular signaling molecules, downstream signaling was thought to be mediated primarily by phosphorylation of signal transducer and activator of transcription (STAT) proteins.[83] STAT proteins are transcription factors activated by members of the Janus kinase (JAK) family[84] of tyrosine kinases involved in intracellular cytokine signaling. A JAK-2 kinase has been shown to bind to the leptin receptor, and when the receptor is activated, JAK-2 phosphorylates one of the STAT proteins, STAT3, which in turn is translocated to the nucleus where it exerts a variety of genomic effects.[85] The time course of such genomic effects is usually measured in hours, however, and cannot explain the relatively rapid neuronal effects of leptin. These include the activation of ATP-sensitive K^+ channels in a subset of hypothalamic neurons that are glucose responsive.[86,87] The possibility that PI-3 kinase might be involved in such a rapid signaling system was recently tested in vivo by blocking neuronal PI-3 kinase signaling with a specific PI-3 kinase inhibitor and demonstrating complete prevention of leptin-induced anorexia as compared with control animals given a vehicle injection. Moreover, the same PI-3 kinase inhibitor did not alter the effect of α-MSH to suppress food intake.[88] Thus, cross-talk between intracellular signaling via the leptin receptor and the insulin receptor may occur at the

level of PI-3 kinase, although each receptor exerts other, independent mechanisms for its neuronal signaling as well (Figure 2).

Summary and Implications for Therapy of Obesity and Disease-related Cachexia

It is now clear that body fat stores are regulated largely by the ability of neural and circulating hormonal inputs to control discrete hypothalamic and hindbrain circuits that regulate energy intake and autonomic function. What becomes apparent is that the activity of the system without these afferent inputs would lead to a persistent drive to eat and store calories. Therefore, obesity can arise from defective input from these key afferent signals—either too little signal is made or the neuronal response to the signal is impaired. The converse may also be true. Thus, evidence is beginning to suggest that excessive leanness can result from excessive satiety signaling or from the activation of catabolic neuronal pathways by signals (e.g., melanocortins) generated as a result of pathologic processes.

The primary focus of this review has been on two of the most carefully characterized and well understood peripheral signals that circulate in proportion to body adiposity stores, insulin, and leptin. Each appears to be taken up into the CNS by a receptor-mediated mechanism, and to act upon receptors that, although present widely throughout the CNS, are particularly abundant in the ventral hypothalamus. Each has been shown to suppress food intake when given peripherally or into the CNS, via mechanisms that do not involve aversive qualities or nonspecific behavioral suppression. Both have been shown to regulate complementary anabolic and catabolic neuronal systems which, when activated, regulate food intake and energy expenditure in a highly coordinated fashion. Impaired neuronal signal transduction by either hormone is associated within increased food intake and body fat stores, and both have been shown to be important to the regulation of reproductive capacity.

Rare patients have been described with defective leptin secretion and action leading to obesity, and more commonly, patients are found with defective melanocortin receptor function in association with obesity.[89] Therefore, these systems appear to be active in human pathophysiology. The successful treatment of leptin-deficient patients with exogenous leptin indicates that appropriate therapy based on an understanding of the pathologic cause of the obesity may be expected to lead to effective treatment programs. However, most patients appear to be leptin resistant, and the etiology of leptin resistance remains to be understood. Nonetheless, potential targets for drug therapy have been identified, based on the signaling peptides already identified (e.g., NPY, AgRP, α-MSH, and CART). Blocking the anabolic molecules might be expected to produce the same therapeutic effect. The recognition of downstream neuronal circuits containing MCH, orexin, TRH, oxytocin, and others likely to be identified, suggests that the number of possible drug targets will increase over time. The potential importance of these secondary targets is highlighted by the observation that knockout of MCH produces an excessively lean animal, raising the possibility that MCH antagonists will produce similar effects. The MCH receptor has been identified and therefore is available for screening. Similarly, attempts to develop antagonists to NPY-5 receptors are underway.

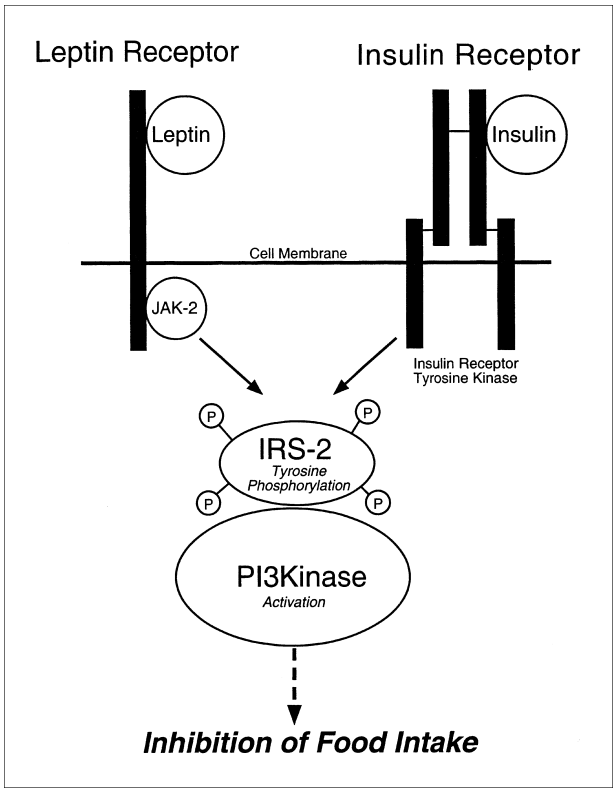

Figure 2. Hypothesized "cross-talk" between insulin and leptin intracellular signaling via the enzyme, phosphotidylinositol 3-kinase (PI3Kinase). Leptin binds to the long form of the leptin receptor ("ObRb"), causing the association of the tyrosine kinase enzyme, Janus kinase-2 (JAK-2) with the cytoplasmic domain of the leptin receptor. Insulin binds to the extracellular alpha subunits of the insulin receptor, activating the tyrosine kinase activity of the intracellular beta subunits. The insulin receptor tyrosine kinase catalyzes the tyrosine phosphorylation the insulin receptor substrate-2 (IRS-2) protein, which associates with PI3Kinase, resulting in activation of PI3Kinase enzyme activity and downstream signal transduction events in neuronal pathways that result in the inhibition of food intake. It is hypothesized that JAK-2 acts through a similar, IRS-dependent mechanism to activate PI3Kinase. Both the leptin receptor and insulin receptor interact with numerous other signal transduction pathways that are not shown but which also could modify food intake and body weight.

Conclusion

The key point is that as any single signal is increased or decreased, the system has the ability to compensate and tends to maintain stability. Insulin and leptin are unique among the many signaling molecules involved because they are critical sensing molecules to the fundamental feedback loop and may therefore need to be affected to produce success in the long term. Alternatively, therapies that simultaneously exert effects on multiple CNS targets could produce the desired outcome in the long term, although in this case, multiple discrete interventions might be required.

Potential long-term complications of such a strategy, a molecular approach that causes body weight to be regulated independently of internal regulatory processes, should also be borne in mind, however. This regulatory system has evolved over many eons and is absolutely essential for life. Recent work in lower organisms such as *C elegans* indicates that neuronal regulation of body fat storage systems via insulin-like signals is critically involved in the regulation of life span as well as nutrient storage and fertility.[90–93] Therefore, it will be important to maintain a broad perspective when evaluating various therapeutic approaches, despite the profound impact of obesity and cachexia on human health in the modern era.

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