Nature

Volume 404(6778) 6 April 2000 pp 661-671

Central nervous system control of food intake

[Insight Review Article]

Schwartz, Michael W.*; Woods, Stephen C.†; Porte, Daniel Jr*; Seeley, Randy J.†; Baskin, Denis G.*‡

Departments of Medicine* and Biological Structure‡, Harborview Medical Center and VA Puget Sound Health Care System, University of Washington, Seattle, Washington 98104-2499, USA
†Department of Psychiatry, University of Cincinnati, Cincinnati, Ohio 45267-0559, USA

Outline

Graphics

Abstract

New information regarding neuronal circuits that control food intake and their hormonal regulation has extended our understanding of energy homeostasis, the process whereby energy intake is matched to energy expenditure over time. The profound obesity that results

in rodents (and in the rare human case as well) from mutation of key signalling molecules involved in this regulatory system highlights its importance to human health. Although each new signalling pathway discovered in the hypothalamus is a potential target for drug development in the treatment of obesity, the growing number of such signalling molecules indicates that food intake is controlled by a highly complex process. To better understand how energy homeostasis can be achieved, we describe a model that delineates the roles of individual hormonal and neuropeptide signalling pathways in the control of food intake and the means by which obesity can arise from inherited or acquired defects in their function.

For most of us, the composition and amount of food that we eat varies considerably from one meal to the next and from one day to the next. Our common experience, therefore, seems at odds with the hypothesis that food intake is highly regulated. Emotions, social factors, time of day, convenience and cost are but a few of the variables that are not biologically regulated, but nonetheless affect meal-to-meal energy intake. As a consequence, daily energy intake is variable both within and among individuals, and is not well correlated with daily energy expenditure 1. Despite short-term mismatches in energy balance, however, most of us match cumulative energy intake to energy expenditure with great precision when measured over a period that spans many meals 1. This phenomenon reflects an active regulatory process, termed energy homeostasis, that promotes stability in the amount of body energy stored in the form of fat.

Although it is overly simplistic to reduce a behaviour as complex as feeding to a series of molecular interactions, discoveries over the past few years have identified signalling molecules that affect food intake and that are critical for normal energy homeostasis. The application of molecular genetics to mice has been especially important in this effort. For example, several monogenic forms of human obesity were identified by searching for mutations homologous to those causing obesity in mice 2-5. Although such monogenic obesity syndromes are rare (see review by Barsh *et al.,* pp. 644-651), the successful use of murine models to study human obesity indicates that substantial homology exists across mammalian species in the functional organization of the weight-regulatory system. More importantly, the identification of molecules that control food intake has generated new targets for drug development in the treatment of obesity and related disorders. Optimism that we may soon enter an era of improved obesity treatment, therefore, seems justified.

Because of the enormous toll on human health taken by obesity and related disorders, an improved understanding of the control of food intake is an important priority. However, the growing number of molecules implicated in energy homeostasis raises nearly limitless possibilities for how body-weight regulation might occur. The aim of this article is to review these advances and to present them in the context of a model for long-term maintenance of energy homeostasis.

Model for energy homeostasis

The increase of food intake (hyperphagia) triggered by a period of fasting is a simple but compelling example of food-intake regulation. The consequent recovery of lost body weight to baseline values, accompanied by the gradual return to normal levels of energy intake 6, is testimony to a regulatory process that is both precise and robust. To explain this phenomenon, Kennedy proposed 7 in 1953 that inhibitory signals generated in proportion to body fat stores act in the brain to reduce food intake. Thus, when weight loss induced by caloric restriction reduces the level of these inhibitory signals, food intake increases until the energy deficit is corrected. This model, however, does not explain how energy intake is controlled during individual meals. Twenty years later, Gibbs and Smith proposed 8 that

signals generated during a meal (termed 'satiety factors'), including peptides secreted from the gastrointestinal tract, provide information to the brain that inhibits feeding and leads to meal termination. A model that seems to unite these two hypotheses is illustrated schematically in Fig. 1.

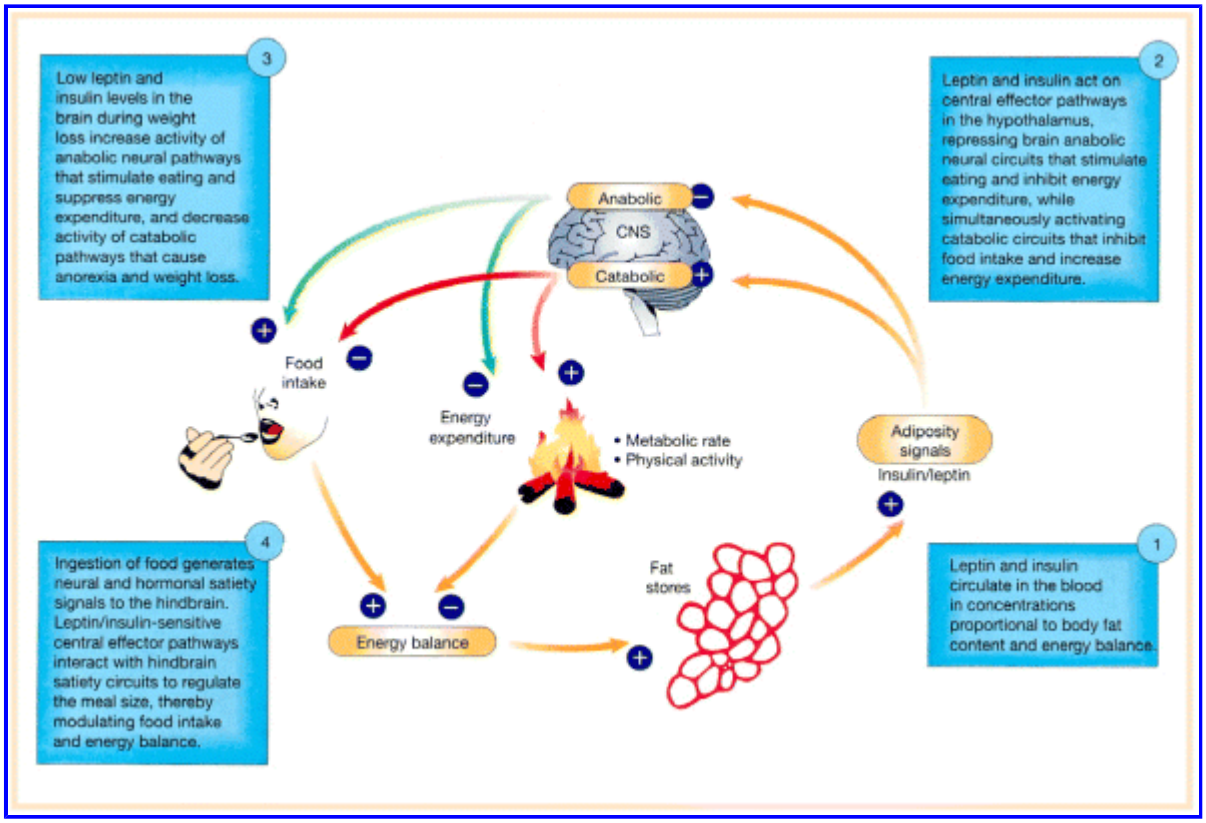


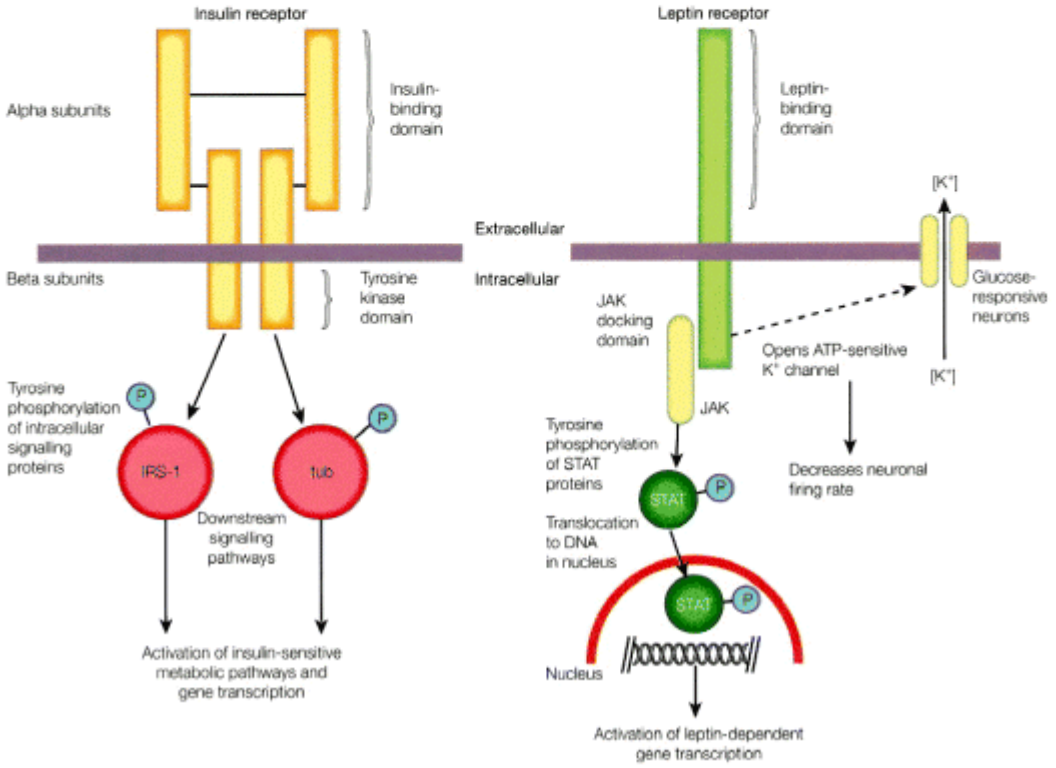
Figure 1 Model showing how a change in body adiposity is coupled to compensatory changes of food intake. Leptin and insulin are adiposity signals, secreted in proportion to body fat content, which act in the hypothalamus to stimulate catabolic, while inhibiting anabolic, effector pathways. These pathways have opposing effects on energy balance (the difference between calories consumed and energy expended) that in turn determines the amount of body fuel stored as fat.

Adiposity signals: leptin and insulin

The pancreatic hormone, insulin, which enters the brain from the circulation 9 and acts there to reduce energy intake 10, was the first hormonal signal to be implicated in the control of body weight by the central nervous system (CNS). The subsequent demonstration that the profound hyperphagia and obesity of *ob/ob* mice results from autosomal recessive mutation of the gene encoding leptin 11, a hormone secreted by adipocytes, provided compelling evidence of a second adiposity signal. Subsequent studies demonstrated that both insulin and leptin fulfil criteria that should be met by any candidate adiposity signal. Both hormones circulate at levels proportional to body fat content 12,13 and enter the CNS in proportion to their plasma level 9,14. Leptin receptors and insulin receptors (see Box 1) are expressed by brain neurons involved in energy intake 15-17, and administration of either peptide directly into the brain reduces food intake 10,18,19, whereas deficiency of either hormone does the opposite 11,20. To date, insulin and leptin are the only molecules that fulfil these criteria.

The insulin receptor comprises an extracellular α-subunit involved in ligand binding and an intracellular β-subunit that transduces the insulin signal to the cell (Box 1 Figure). The β-subunit has intrinsic tyrosine kinase activity that, upon insulin binding, activates intracellular signalling proteins by phosphorylating them on tyrosine residues[115]. These proteins include insulin-receptor substrate (IRS)-1, which is present in neurons and in peripheral tissues[116]. The neuronal protein encoded by the *Tub* gene is also tyrosine-phosphorylated in response to activation of the insulin receptor[117]. Because a loss-of-function mutation of *Tub* causes obesity in *tubby* mice, there is new support for the hypothesis that defective insulin signalling in the brain causes obesity.

Unlike insulin receptors, leptin receptors are members of the cytokine-receptor superfamily and do not have intrinsic tyrosine kinase activity[118]. However, the leptin receptor does have docking sites for janus kinases (JAK), a family of tyrosine kinases involved in intracellular cytokine signalling[119]. Activated JAK phosphorylates members of the signal transduction and transcription (STAT) family of intracellular proteins. STAT proteins, in turn, stimulate transcription of target genes that mediate some of leptin's cellular effects. Although regulation of hypothalamic neuropeptide gene expression by leptin is well described, the role of JAK–STAT signalling in this response remains uncertain, as leptin can affect neuronal firing rate independently of its transcriptional effects. For example, a subset of 'glucose-responsive' neurons in the hypothalamus become hyperpolarized (and therefore decrease their firing rate) within minutes of leptin application[120]. Glucose influences the membrane potential of glucose-responsive neurons indirectly through its oxidation to generate ATP, which in turn controls the activity of ATP-sensitive potassium channels (K_{ATP}) in the plasma membrane[121]. Closure of K_{ATP} channels in response to increasing intracellular concentrations of ATP (relative to ADP) raises intracellular [K^+], which depolarizes the cell and increases its firing rate. Because leptin maintains K_{ATP} channels in the open configuration[120], positively charged K^+ ions diffuse out of the cell and lower its membrane potential. This effect on K_{ATP} channels can be detected even in isolated patches of plasma membrane, excluding a mechanism involving transcriptional effects of leptin. The relationships among leptin signalling through the JAK–STAT pathway, its effects on neuronal firing rate, and its control of neuropeptide gene expression are an important area for future study.



Box 1 Leptin- and insulin-receptor signalling

Different mechanisms underlie the association of insulin and leptin with body fat content 21. The effect of weight gain to reduce insulin sensitivity seems to explain how insulin, but not leptin, varies according to body fat stores 22. As weight increases, insulin secretion must increase in both the basal state and in response to meals to compensate for insulin resistance if normal glucose homeostasis is to be maintained 23,24. Failure of the pancreatic [beta]-cell to achieve this adaptive increase of insulin secretion causes hyperglycaemia, and probably contributes to the association of type 2 diabetes with obesity. Increased insulin secretion as obesity progresses is thus hypothesized to increase insulin delivery to the brain, where it helps to limit further weight gain.

Mechanisms involved in leptin secretion are quite different. The rate of insulin-stimulated glucose utilization in adipocytes is a key factor linking leptin secretion to body fat mass 25. Although the mechanism is incompletely understood, it may involve glucose flux through the hexosamine pathway 26. Because acute changes of energy balance markedly affect adipocyte

glucose metabolism, leptin secretion can become transiently dissociated from levels of total body fat. For example, food deprivation lowers plasma leptin concentrations in both rodents and humans much more rapidly and to a greater extent than would be expected from the decrease of body fat content. This exaggerated early decline of leptin levels would enable compensatory responses to be activated before energy stores are substantially depleted.

Several observations indicate that leptin has a more important role than insulin in the CNS control of energy homeostasis. For example, leptin deficiency causes severe obesity, with hyperphagia that persists despite high insulin levels. In contrast, obesity is not induced by insulin deficiency. But such comparisons are complicated by the critical role that insulin has in promoting both fat storage and leptin synthesis by fat cells. Because fat deposition requires insulin, weight gain cannot occur when insulin deficiency is present, even if food is consumed in large amounts. For example, in uncontrolled diabetes mellitus (the disease induced by the loss of insulin), food intake increases markedly 27, but levels of both body adiposity and plasma leptin remain low in rats 28 and humans 29. Rather than being stored as fat, excess calories ingested in this context contribute to elevated blood glucose levels, and ultimately, much of this glucose is lost in the urine. Because both insulin and leptin levels are low in this type of diabetes, the long-recognized syndrome of 'diabetic hyperphagia'27 could potentially result from reduced CNS signalling by insulin, leptin, or by both hormones. A recent study sought to clarify this issue by selectively replenishing leptin (but not insulin) to nondiabetic levels through exogenous leptin infusion in a rat model of uncontrolled, insulin-deficient diabetes 30. Because this intervention prevented the development of diabetic hyperphagia, it was concluded that deficiency of leptin, but not insulin, is required for hyperphagia in this model. Thus, although both leptin and insulin probably participate in the CNS control of energy homeostasis, available data indicate that leptin has the more critical role.

Leptin resistance and obesity

The hypothesis that leptin resistance can occur in association with obesity was first suggested by the finding of elevated plasma leptin levels in obese humans 13. This hypothesis suggests that some cases of human obesity may be due to reduced leptin action in the brain, and that affected individuals are unlikely to respond to pharmacological treatment with leptin. Resistance to leptin is clearly documented in mice (for example, *db/db*)19 and rats (for example, *fa/fa*)31 bearing mutant leptin receptors, but also in mice that develop obesity for other reasons. These include mice with genetic ablation of thermogenic brown adipose tissue 32, mice that lack melanocortin-4 (MC4) receptors 33, agouti (A^y/a) mice 34 (see later) and mice fed a highly palatable high-fat diet 19.

Several mechanisms may contribute to leptin resistance. By decreasing the ability of circulating leptin to enter brain interstitial fluid, where it can bind to neuronal leptin receptors, impaired leptin transport across endothelial cells of the blood-brain barrier is one potential mechanism. Several studies indicate that, like insulin 9, leptin uptake into the brain is facilitated by leptin receptors expressed by endothelial cells 35 in the blood-brain barrier that function as leptin transporters. Whether dysfunction of this transport process can lead to obesity remains to be determined, but the finding that obese humans have leptin levels in cerebrospinal fluid that are low in comparison to plasma 36 is consistent with this possibility.

Reduced leptin-receptor signal transduction is another potential cause of leptin resistance. This has been documented not only in the brain of rodents bearing mutant leptin receptors, but also as an acquired response to leptin-receptor activation. Like some other cytokine receptors, activation of the leptin receptor induces expression of a protein that inhibits further leptin signal transduction, termed 'suppressor of cytokine signalling-3' (SOCS-3)37. The potential contribution of SOCS-3 to acquired forms of leptin resistance and obesity is an

active area of study.

Upon activation of leptin receptors in the brain, a series of integrated neuronal responses is probably required for food intake and energy balance to be affected. Failure of one or more neuronal systems in this circuit to respond to the leptin signal will therefore manifest as leptin resistance. The key role that these neuronal effector pathways have in energy homeostasis makes them an important priority for study and is the focus of the following discussion.

Neuropeptide effectors of adiposity signals

Several distinct hypothalamic neuropeptide-containing pathways have emerged as candidate mediators of leptin and insulin action in the CNS (Table 1).

Molecule	Regulation by adiposity signals
Orexigenic	
NPY*	↓
AGRP*	↓
MCH	↓
Hypocretin 1 and 2/orexin A and B	↓
Galanin	?
Noradrenaline	?
Anorexigenic	
α-MSH*	↑
CRH*	↑
TRH*	↑
CART*	↑
IL-1β*	↑
Urocortin*	?
Glucagon-like peptide 1	?
Oxytocin	?
Neurotensin	?
Serotonin	?

Orexigenic refers to molecules that promote increased energy intake; anorexigenic implies the opposite. An asterisk designates documented, coordinated effects on both food intake and energy expenditure that promote a change in energy stores; arrows designate direction of effect exerted by one or both of the adiposity signals, leptin and insulin.

Table 1 Neuropeptides implicated in the control of energy homeostasis

Neuropeptide Y stimulates food intake

Prominent among anabolic effector pathways is a circuit containing neuropeptide Y (NPY). Injection of NPY into cerebral ventricles or directly into the hypothalamus of rats potently stimulates food intake 38 and decreases energy expenditure while simultaneously inducing lipogenic enzymes in liver and white adipose tissue 39. Consequently, continuous or repeated central administration of NPY leads readily to obesity 38,40. Because NPY gene expression and secretion of the NPY peptide in the hypothalamus are increased during active depletion of body fat stores 41,42 and/or reduced leptin/insulin signalling to the brain 43, NPY meets the criteria for an anabolic signalling molecule. Moreover, leptin inhibits arcuate nucleus NPY gene expression 44,45 and genetic knockout of NPY reduces hyperphagia and

obesity in *ob/ob* mice 46, indicating that the full response to leptin deficiency requires NPY signalling (Fig. 2a). The hyperphagic response to insulin-deficient diabetes is similarly accompanied by increased hypothalamic NPY synthesis and release 47, and this response is blocked by insulin administration, either systemically or directly into the brain 20. The finding that mice that lack NPY (but are otherwise genetically normal) have intact feeding responses, however, raises questions about the need for NPY when leptin or insulin levels are normal 48. Alternatively, congenital absence of a major neuropeptide such as NPY may elicit compensatory responses that mask the consequences of its deficiency, and further study is required to resolve this issue. Agouti-related protein (AGRP), orexin (also known as 'hypocretin') and melanin-concentrating hormone (MCH) have subsequently been added to the list of candidate anabolic effector signalling molecules (Table 1).

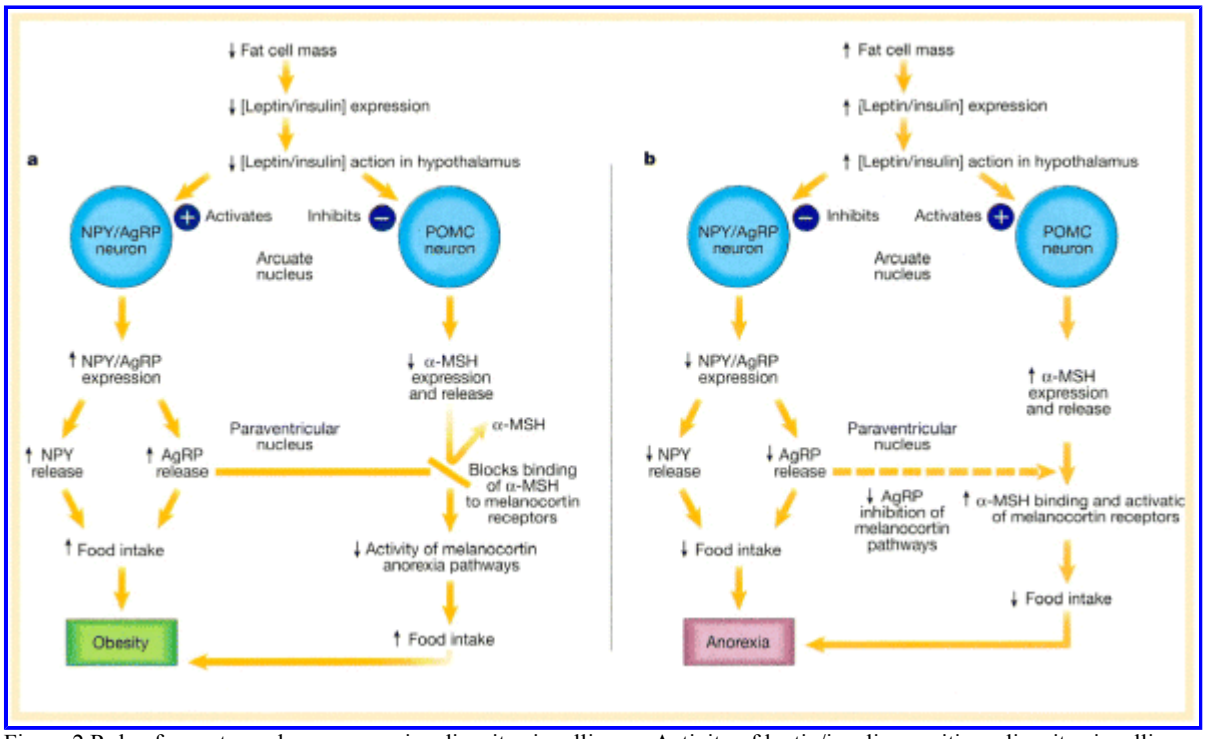


Figure 2 Role of arcuate nucleus neurons in adiposity signalling. **a,** Activity of leptin/insulin-sensitive adiposity signalling pathways in hypothalamus under conditions of leptin/insulin deficiency. **b,** Increased action of leptin/insulin in arcuate nucleus inhibits the NPY/AGRP anabolic pathway and stimulates the POMC catabolic pathway, resulting in reduced food intake and anorexia.

Melanocortins suppress food intake

Candidate catabolic effector signalling molecules have an opposite set of characteristics. Melanocortins such as [alpha]-melanocyte-stimulating hormone ([alpha]-MSH)49, as well as corticotropin-releasing hormone (CRH)50, thyrotropin-releasing hormone (TRH)51, cocaine- and amphetamine-regulated transcript (CART)52 and interleukin-1[beta]53 are among a growing list of peptides that promote negative energy balance. Neuronal synthesis of these peptides increases in response to increased adiposity signalling in the brain. Among these, the melanocortin system stands out as being remarkable both for its complexity and its importance to energy homeostasis.

Melanocortins are peptides (such as [alpha]-MSH) that are cleaved from the pro-opiomelanocortin (POMC) precursor molecule and that exert their effects by binding to members of a family of melanocortin receptors 49. A role for melanocortin signalling in the control of energy homeostasis first emerged after the cloning of the MC3- and MC4-receptor genes and the demonstration that they are expressed primarily in the brain 54. This discovery was followed by evidence that a synthetic agonist of these receptors suppresses food intake,

whereas a synthetic antagonist has the opposite effect 55. The report that mice lacking the MC4 receptor (owing to gene targeting) are hyperphagic and very obese 56 indicates that tonic signalling by MC4 receptors limits food intake and body fat mass. Mice heterozygous for the deleted MC4 allele also become obese, although less so than homozygous knockouts 56. Lack of a full complement of central MC4 receptors, therefore, predisposes to hyperphagia and pathological weight gain. This finding has since been extended to humans with MC4-receptor mutations 4,5.

Further evidence for the importance of melanocortin signalling came from studies of agouti (A^y/a) mice, an autosomal dominant model of genetic obesity characterized by a yellow coat colour and an obese phenotype. Cloning of the *agouti* gene 57 identified a protein ('agouti') that functions as an antagonist of cutaneous MC1 receptors and normally is expressed by hair follicles. By reducing MC1 signalling, increased cutaneous agouti lightens the coat colour. Agouti mice, however, express agouti in tissues throughout the body and consequently develop both a yellow coat colour and obesity (owing to ectopic agouti production within the brain, where it antagonizes MC4 receptors)49 (Fig. 2b).

The subsequent cloning of the *Agrp* gene 58 identified a peptide, AGRP, with homology to *agouti* that is an antagonist of MC3 and MC4 receptors 59. The demonstration that hypothalamic AGRP expression, like that of NPY and POMC, is localized to the arcuate nucleus 58, and that it is upregulated by fasting 60,61 and by leptin deficiency 58, indicates that antagonism of CNS melanocortin receptors is important in body-weight regulation. Consistent with its role as an anabolic signalling molecule, AGRP causes hyperphagia when administered intracerebroventricularly (i.c.v.)62,63 or expressed transgenically 59, and the increase of food intake following a single i.c.v. injection of AGRP is sustained for up to a week 62. Although NPY is described as the most potent orexigenic molecule (that is, a molecule that stimulates increased energy intake) when the feeding response is measured over a few hours, its effects are short-lived in comparison to those of AGRP. AGRP must therefore be considered the most robust orexigenic molecule if potency is measured as the cumulative increment of energy intake after a single i.c.v. injection. The mechanism underlying the extraordinary duration of action of AGRP remains a fascinating area for further investigation.

Neuropeptide signalling pathways in the hypothalamus

Brain lesioning and stimulation studies performed some six decades ago first implicated the hypothalamus as a major centre controlling food intake and body weight (Fig. 3). As summarized in a classic paper by Stellar 64, these studies identified the ventromedial hypothalamic nucleus (VMN) as the 'satiety centre', while the lateral hypothalamic area (LHA) was termed the 'hunger centre' (reviewed in ref. 50). These designations reflected the ability of electrical stimulation of the VMN to suppress food intake, and of bilateral VMN lesions to induce hyperphagia and obesity. Conversely, stimulation or lesioning of the LHA induced the opposite set of responses. As our knowledge of specific neuronal subpopulations involved in energy homeostasis has expanded, the notion of specific 'centres' of the brain that control food intake and body weight has been replaced by that of discrete neuronal pathways that generate integrated responses to afferent input related to changing body fuel stores 65.

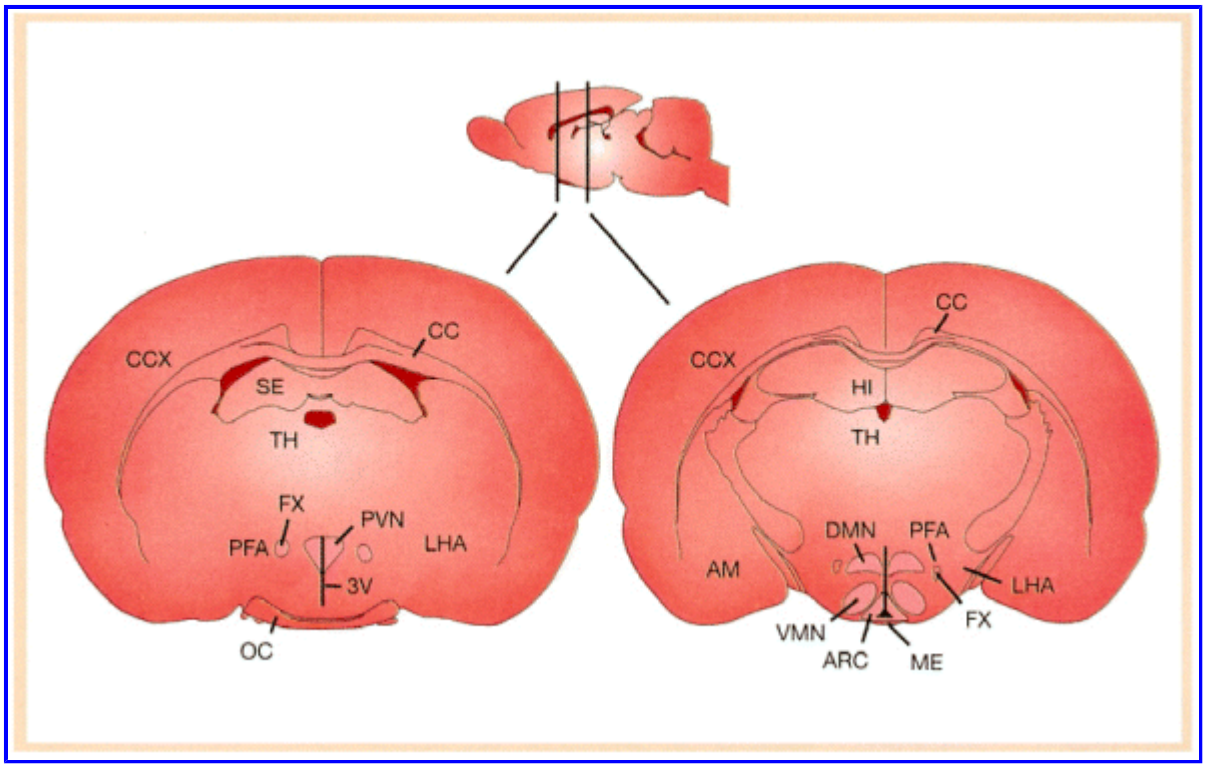


Figure 3 Diagrams of rat brain, showing major hypothalamic regions implicated in adiposity signalling and regulation of food intake. The small figure at the top is a longitudinal view of a rat brain, with olfactory bulb at the anterior end on the left and the caudal hindbrain on the right. Cross-sections of the brain at two levels (indicated by vertical lines) are shown at the left and right. First-order neurons responding to adiposity signals are located in the arcuate nucleus (ARC) and project anteriorly to the PVN as well as the PFA adjacent to the fornix (FX) and the LHA. Other regions implicated in regulating food intake include the ventromedial nucleus (VMN) and dorsomedial nucleus (DMN). Abbreviations of brain structures: AM, amygdala; CC, corpus callosum; CCX, cerebral cortex; HI, hippocampus; ME, median eminence; OC, optic chiasm; SE, septum; TH, thalamus; 3V, third ventricle.

Transduction of adiposity signals into a neuronal response

Situated adjacent to the floor of the third ventricle, the arcuate nucleus is an elongate ('arc-like') collection of neuronal cell bodies occupying approximately one-half of the length of the hypothalamus. NPY and AGRP are co-localized in arcuate nucleus neurons 60,61, demonstrating that a single neuronal cell type can contain multiple anabolic effector molecules. The subsequent finding that POMC and CART are co-localized in a distinct, but adjacent, subset of arcuate nucleus neurons 66 indicates that circuits originating in this brain area have highly specialized roles in energy homeostasis (Fig. 4).

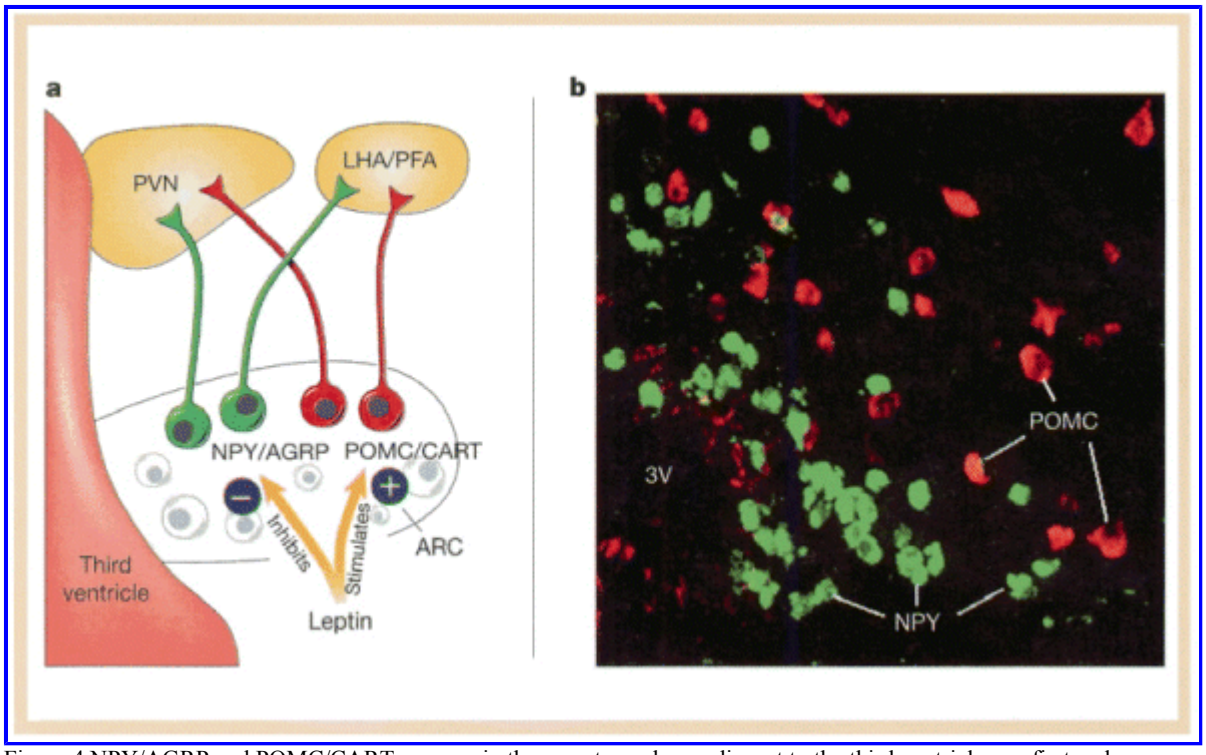


Figure 4 NPY/AGRP and POMC/CART neurons in the arcuate nucleus, adjacent to the third ventricle, are first-order neurons in the hypothalamic response to the circulating adiposity signals insulin and leptin. **a,** Populations of first-order NPY/AGRP (green) and POMC/CART (red) neurons in the arcuate nucleus (ARC) are regulated by leptin and project to the PVN and to the LHA and PFA, which are locations of second-order hypothalamic neuropeptide neurons involved in the regulation of food intake and energy homeostasis. **b,** Fluorescence *in situ* hybridization detection of mRNAs encoding NPY (green cells) and POMC (red cells) in the arcuate nucleus, adjacent to the third ventricle (3V). NPY and POMC are expressed in discrete populations of arcuate nucleus neurons. NPY release in the PVN and LHA/PFA regions stimulates eating, whereas release of [alpha]-MSH (derived from POMC) in the PVN has an anorexic effect.

The hypothesis that the arcuate nucleus transduces information related to signalling by leptin into a neuronal response is supported by the anorexic response to local microinjection of leptin into this area 67, and the inability of i.c.v. leptin to reduce food intake after the arcuate nucleus has been destroyed 68,69. A majority of both NPY/AGRP and POMC/CART neurons have been found to coexpress leptin receptors 16,17 and both types of neurons are regulated by leptin (as judged by changes in neuropeptide gene expression), but in an opposing manner. Thus, NPY/AGRP neurons are inhibited by leptin, and consequently are activated in conditions where leptin levels are low 44,45,60,61. Although less well studied, a deficiency of insulin also seems to activate these neurons 20,47, and insulin receptors are highly concentrated in the arcuate nucleus 15. Conversely, conditions characterized by reduced insulin or leptin inhibit POMC 70,71 and CART 52 expression in the arcuate nucleus, and administration of these hormones can prevent or attenuate these neuropeptide responses. Moreover, involuntary overfeeding in rats, which potently inhibits spontaneous food intake once body weight has increased by more than 5%, elicits a threefold increase of POMC messenger RNA levels in the arcuate nucleus 72. The demonstration that anorexia induced either by leptin 73 or by involuntary overfeeding 72 is reversed by central administration of a melanocortin-receptor antagonist (at a low dose that has no effect on food intake in control animals) indicates that melanocortin signalling is a mediator of the anorexic response induced by increased adiposity signalling to the brain. Taken together, these findings indicate that the arcuate nucleus is a major site for transducing afferent input from circulating leptin and insulin into a neuronal response.

Implicit in this hypothesis is the suggestion that brain areas innervated by arcuate nucleus neurons are sites where second-order neurons involved in the energy homeostasis circuit are located. But the identification of such downstream neurons is just beginning, and energy homeostasis probably involves integrated and redundant pathways, rather than a discrete set

of neurons connected in series to one another. Models for understanding how arcuate nucleus neurons ultimately affect food intake nonetheless provide a useful framework for future study. One possible model is proposed below.

Model for second-order neuronal signalling pathways

Hypothalamic areas including the paraventricular nucleus (PVN), zona incerta, perifornical area (PFA) and LHA are richly supplied by axons from arcuate nucleus NPY/AGRP and POMC/CART neurons [74,75]. Insight into the role in energy homeostasis played by neurons in these areas can be gleaned from earlier stimulation and lesioning studies. For example, PVN stimulation inhibits food intake, whereas the reverse is true of stimulation of the LHA [50] and adjacent PFA [76]. Conversely, bilateral PVN lesions cause a hyperphagic obesity syndrome, whereas bilateral lesioning of the LHA causes anorexia and weight loss [50,64]. These observations indicate that anorexigenic and orexigenic signalling molecules might be synthesized in the PVN and LHA, respectively (Fig. 5).

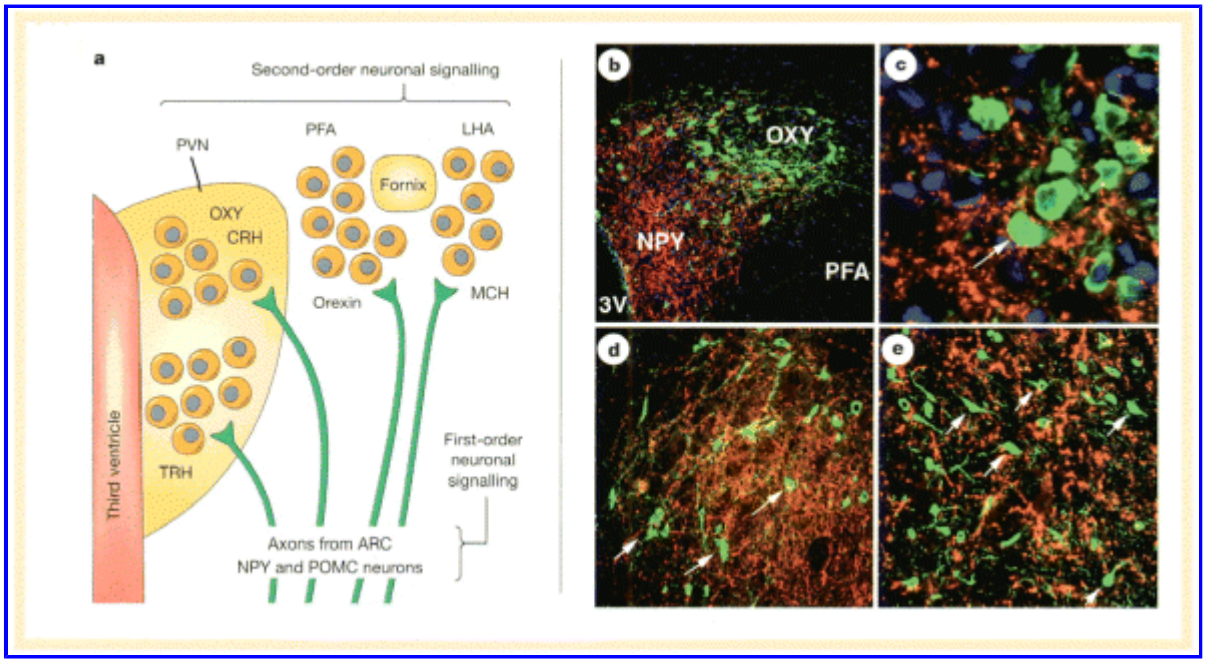


Figure 5 Locations of candidate second-order neurons involved in the hypothalamic response to insulin and leptin adiposity signalling. **a,** Diagram showing neuronal axons containing NPY and [alpha]-MSH from the arcuate nucleus (ARC) innervating the PVN, LHA and PFA (adjacent to the fornix). Candidate second-order neurons include those that express TRH, CRH and oxytocin (OXY) in the PVN (which cause anorexia), and neurons that express orexins and MCH in the PFA and LHA (which increase feeding). **b,** Fluorescence double-immunocytochemical staining of the PVN, showing NPY-containing axons (red fluorescence) in the parvocellular region adjacent to the third ventricle and magnocellular OXY neurons (green fluorescence) in the lateral PVN. The PFA is located lateral to the PVN, but the fomix and LHA are not included in this field. **c,** Higher magnification of OXY neurons (green fluorescence) surrounded by axons and terminals containing NPY (red fluorescence). Nuclei of cells are shown in blue fluorescence. **d,** PFA showing NPY-containing axons (red fluorescence) surrounding neuron cell bodies containing orexins (green fluorescence). **e,** LHA showing NPY-containing axons (red fluorescence) surrounding neuron cell bodies containing MCH (green fluorescence).

Consistent with these predictions, several neuropeptides synthesized in PVN neurons reduce food intake and body weight when administered centrally. These include CRH, which causes anorexia and also activates the sympathetic nervous system in addition to its role as a major regulator of the hypothalamic-pituitary-adrenal axis [50,77]; TRH, which reduces food intake [51] in addition to stimulating the thyroid axis; and oxytocin, which reduces food intake in addition to regulating uterine function [78]. If these PVN neurons are second-order catabolic effectors located downstream of the arcuate nucleus, they should be stimulated by melanocortin and/or CART signalling, but inhibited by NPY signalling, and further study is

warranted to test this prediction.

The hypothesis that second-order neurons involved in anabolic signalling reside within the LHA/PFA is supported by studies of MCH, an orexigenic peptide located in this brain area 79. Evidence that MCH synthesis is elevated by both energy restriction and leptin deficiency 79, and that MCH-knockout mice have reduced food intake and are excessively lean 80, is consistent with this model. The discovery of the MCH receptor as a G-protein-coupled receptor (previously known as SLC-1)81,82 also supports the hypothesis of MCH as an orexigenic factor. Like NPY receptors, the MCH receptor is coupled to the G_i subunit of the plasma membrane G-protein assembly. By activating G_i, binding of MCH to its receptor inhibits formation of cyclic AMP and consequently reduces signalling by protein kinase A (PKA)81,82. This effect is opposite to that mediated by activation of receptors that exert anorexic effects, such as MC4 or CRH receptors, which are coupled to G_s and consequently increase cAMP and PKA signalling.

Two additional peptides are expressed exclusively in the LHA, zona incerta and PFA. Termed 'hypocretins 1 and 2'83 or 'orexins A and B'84 by the two groups that simultaneously discovered them, these peptides increase food intake and cause generalized behavioural arousal when administered centrally 84,85. Targeted deletion of the hypocretin/orexin gene in mice induces narcolepsy 86, a disorder characterized by the sudden onset of sleep at times when it would not ordinarily occur. This finding indicates that reduced hypocretin/orexin signalling may contribute to the onset and maintenance of sleep, in addition to its potential role in the control of food intake. Integration of MCH and hypocretin/orexin neurons into a model of the hypothalamic pathways controlling energy homeostasis predicts that they should be inhibited by melanocortin or CART input, and stimulated by NPY signalling, from neurons of the arcuate nucleus.

Much work must be done to test this model of first- and second-order neurons in the energy homeostasis circuit. Identifying specific neuronal subsets in the PVN and LHA that express NPY and melanocortin receptors is an important priority. Because many neurons of the PVN, PFA and LHA project to the arcuate nucleus, neuronal traffic flows bidirectionally between the arcuate nucleus and these other hypothalamic sites. So rather than being passive recipients of information from the arcuate nucleus, these second-order neurons can actively modify the information that arrives there. In addition, leptin receptors have been described on PVN and LHA neurons, implicating them as direct targets for regulation by circulating adiposity signals. However, far greater concentrations of leptin receptors are present in the arcuate nucleus than in these other hypothalamic sites.

Satiety signals control meal size

It is self-evident that either the amount of food consumed during individual meals, the frequency of meals, or both, must be regulated if energy homeostasis is to be achieved. The major determinant of meal size is the onset of satiety, a biological state induced by neurohumoral stimuli generated during food ingestion that leads to meal termination. To clarify how the decision to terminate a meal once it has begun is controlled in the regulation of energy homeostasis, we propose that hypothalamic pathways involved in energy homeostasis interact with a distinctly different set of pathways involved in the response to satiety signals 65,87. In contrast to the timing of meal initiation, which can be influenced by many external and internal variables (for example, emotional factors, time of day, availability and palatability of foods, and threats from the environment), meal termination tends to be a more biologically controlled process 88. Several findings indicate that control of meal size is a component of the feeding response induced by changes of body fuel stores or adiposity signalling. The hyperphagic response to central administration of NPY, for example, arises predominantly from the consumption of larger meals 89. Conversely, leptin-treated animals

consume the same number of meals as vehicle-treated controls, but the meals are smaller 90. These observations indicate that signals involved in energy homeostasis may control food intake primarily by adjusting the size of individual meals. One way that this could be accomplished is by modulating the response to satiety signals in brain areas that process this information.

In contrast to its major role in mediating the response to adiposity signals, the hypothalamus is probably not the site that processes satiety signals. Rather, satiety information generated during the course of a meal is largely conveyed to the hindbrain by means of afferent fibres of the vagus nerve and by afferents passing into the spinal cord from the upper gastrointestinal tract 91. This information converges in the nucleus tractus solitarius (NTS), an area of the caudal brainstem that integrates sensory information from the gastrointestinal tract and abdominal viscera, as well as taste information from the oral cavity 92. Satiety-inducing signals that reach the NTS are initiated by mechanical or chemical stimulation of the stomach and small intestine during food ingestion, neural input related to energy metabolism in the liver 93 and humoral signals such as cholecystokinin (CCK) that are released upon nutrient stimulation of neuroendocrine secretory cells lining the intestinal lumen 94. Meal termination induced by such satiety signals can be demonstrated even when all neuronal connections between forebrain and hindbrain are severed 95. The basic process of terminating a meal, therefore, involves brain areas that can function in the absence of hypothalamic influences.

How then is the forebrain response to adiposity signals coupled to changes in the size of single meals? The hypothesis that such responses ultimately involve an interaction with hindbrain areas that control satiety is supported by the ability of both leptin 96 and insulin 87 to enhance the satiating effect of CCK. This interaction may be explained by the ability of central effector pathways to influence the response of NTS neurons to input from vagal afferents that convey satiety-related stimuli (Fig. 6). Recent evidence that leptin potentiates the effect of CCK to activate NTS neurons demonstrates clearly that signals involved in energy homeostasis modulate the response of NTS neurons to input related to satiety 97.

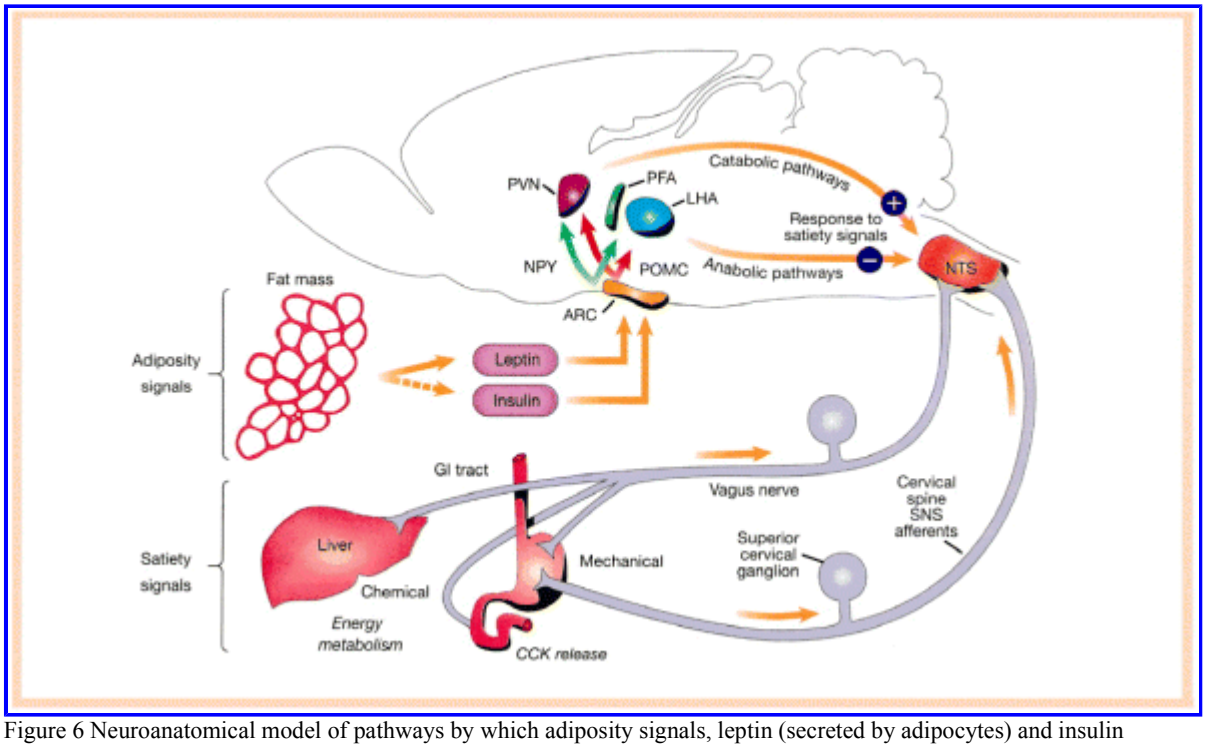


Figure 6 Neuroanatomical model of pathways by which adiposity signals, leptin (secreted by adipocytes) and insulin (secreted by the endocrine pancreas in proportion to adiposity), interact with central autonomic circuits regulating meal size. Leptin and insulin are proposed to stimulate a catabolic pathway (POMC/CART neurons) and inhibit an anabolic pathway

(NPY/AGRP neurons) that originates in the arcuate nucleus (ARC). These pathways project to the PVN and LHA/PFA, where they make connections with central autonomic pathways that project to hindbrain autonomic centres that process satiety signals. Afferent input related to satiety from the liver, gastrointestinal tract and from peptides such as CCK are transmitted through the vagus nerve and sympathetic fibres to the nucleus of the solitary tract (NTS), where they are integrated with descending hypothalamic input. Net neuronal output from the NTS and other hindbrain regions leads to the termination of individual meals, and is potentiated by catabolic projections from the PVN and inhibited by input from the LHA/PFA. Reduced input from adiposity signals (for example, during diet-induced weight loss), therefore, increases meal size by reducing brainstem responses to satiety signals. Not shown are ascending projections from hindbrain to forebrain that may also contribute to adaptive changes in food intake.

Several caveats follow as a result from the hypothesis that NTS neurons are themselves responsible for integrating afferent information related to satiety with descending inputs from forebrain neurons involved in energy homeostasis. First, NTS neurons have reciprocal interconnections with forebrain areas such as the PVN [98], so the integration of satiety and energy homeostasis information probably involves multiple brain areas. In addition, the neuronal substrates for responding to central effector peptides involved in energy homeostasis are present locally within the NTS, as well as in the hypothalamus. For example, MC4 receptors are expressed in the NTS [54], and local administration of MC4-receptor agonists or antagonists into the fourth ventricle (which is adjacent to the NTS) elicits feeding responses that are indistinguishable from those induced by injecting these compounds into the more rostral lateral ventricle [99]. This finding, combined with evidence that leptin receptors [100] and POMC neurons are both present in the NTS (the only brain area other than the arcuate nucleus that expresses the POMC gene)[101], indicates that the hindbrain and forebrain may both process information involved in energy homeostasis. Thus, the NTS or other brainstem areas may, like the arcuate nucleus, contain neurons that respond to leptin and, through ascending projections to key forebrain sites, contribute to adaptive feeding responses to changes in body fat content. Further clarification of the mechanisms that integrate forebrain and hindbrain circuitry involved in this process is an important area for future study.

Monoamine neurotransmitters and food intake

Noradrenaline

Noradrenaline is synthesized in brainstem areas such as the dorsal vagal complex and the locus ceruleus. These areas project both caudally to the spinal cord and rostrally to the hypothalamus, thalamus and cortex. In some of these neurons, including those projecting to the PVN, noradrenaline is co-localized with NPY. Like NPY, injection of noradrenaline into the PVN increases food intake robustly and repeated injections can result in substantial weight gain [102]. The observation of elevated noradrenaline levels in the PVN of *ob/ob* mice [103] indicates that leptin may inhibit noradrenaline release from terminals in this brain area, a possibility supported by *in vitro* studies using rat hypothalamus [104]. Increased noradrenaline signalling in the PVN or other hypothalamic areas may therefore contribute to hyperphagia induced by leptin deficiency, a hypothesis that implicates noradrenaline as an anabolic effector in the CNS control of energy homeostasis.

Dopamine

A critical dependency of food intake on CNS dopamine signalling is implied by the profound feeding deficits that result from both pharmacological depletion [105] and genetic disruption [106] of dopamine synthesis. The interpretation of this finding is complicated, however, by motor impairments associated with dopamine deficiency that may also affect feeding behaviour. The observation that the feeding effects of dopamine vary with the brain region under study further obscures its role in energy homeostasis. For example, mesolimbic dopamine pathways (comprised of cell bodies in the substantia nigra and ventral tegmental area that project to the nucleus accumbens, striatum and cerebral cortex) seem to contribute

to the 'rewarding' aspects of consuming palatable foods 107. In contrast, dopamine signalling in the hypothalamus via neurons situated in the dorsomedial and arcuate nuclei seems to inhibit food intake. Although reduced dopamine levels in the arcuate nucleus of *ob/ob* mice 103 raise the possibility that decreased hypothalamic dopamine signalling contributes to hyperphagia induced by leptin deficiency, the finding that leptin inhibits dopamine release from rat hypothalamus *in vitro*104 is inconsistent with this hypothesis.

Synaptic concentrations of neurotransmitters are determined not only by the rate of their release from nerve terminals, but also by their rate of removal from the synaptic cleft. The latter process is dependent on specific transporter proteins that mediate neurotransmitter reuptake, the expression of which can be influenced by metabolic and hormonal factors 108. For example, fasting and uncontrolled diabetes reduce synaptic dopamine reuptake (which increases synaptic dopamine levels)109, whereas the reverse is true for noradrenaline 108. Because these effects are reversed by insulin infusion directly into the brain, they may be mediated, at least in part, by reduced CNS insulin signalling.

Serotonin

The serotonin system is comprised of cell bodies in the caudal brainstem including the dorsal raphe nuclei that project widely throughout the brain, and is the primary target of several centrally acting drugs developed for obesity treatment (for example, dexfenfluramine and sibutramine). Such drugs increase serotonin-receptor signalling and thereby suppress food intake, whereas antagonists have the opposite effect 110. The $5HT_{2C}$ serotonin-receptor subtype is implicated in this process, as knockout of this receptor increases food intake and body weight 11. Maintenance of normal energy homeostasis, therefore, seems to require intact serotonin signalling. But obesity in this model is modest, especially when compared to the phenotype of mice lacking MC4 or leptin receptors. The recent finding that leptin increases serotonin turnover 112 raises the possibility that at least some of leptin's weight-reducing effects are mediated through increased serotonin signalling. However, leptin-induced anorexia is intact in mice lacking the $5HT_{2C}$ receptor 111, indicating that leptin's ability to reduce food intake does not require signalling at this receptor subtype.

Aminergic neurotransmitter systems, therefore, exert unambiguous effects on food intake and provide important targets for current approaches to drug treatment of obesity. The role of these systems in energy homeostasis is complex, however, and the hypothesis that they serve as major targets for the action of adiposity signals is not strongly supported.

Therapeutic implications

A more detailed understanding of the pathogenesis of human obesity may ultimately guide treatment of affected individuals. Obesity that results from reduced melanocortins, for example, might respond well to administration of melanocortin-receptor agonists, if and when they become available in a clinical setting. Evidence for this is provided in a recent study of POMC-knockout mice, in which obesity was reversed by administration of an MC4-receptor agonist 113. Analogously, administering leptin to an obese human with genetic leptin deficiency reduced weight as markedly as it does in *ob/ob* mice 114. Obesity that is associated with leptin resistance, however, may be common and would be unlikely to respond to leptin treatment unless the resistance can be overcome. Patients with defective melanocortin-receptor function, for example, seem unlikely to respond to therapy with either leptin or melanocortin-receptor agonists. These considerations indicate that an expanded ability to diagnose the pathophysiological basis of human obesity will have direct applications to its treatment. However, a multidrug regimen that targets multiple sites within the weight-regulatory system may be necessary to achieve and sustain weight loss in many individuals.

The impressive effects of AGRP on food intake in rodents 62,63 indicate that it warrants evaluation in the treatment of conditions associated with excessive weight loss, including anorexia nervosa and wasting illness associated with AIDS or cancer. If AGRP proves ineffective in this context, it would indicate that the pathogenesis of such conditions involves pathways additional to, and potentially downstream of, melanocortin-receptor activation. Such an outcome might therefore direct therapeutic strategies towards activation of other candidate anabolic (or inhibition of catabolic) effector pathways.

These considerations highlight the importance of clarifying the mechanisms that control food intake and energy homeostasis. Such information will help us to understand the pathogenesis of disorders at both ends of the body-weight spectrum, and is a probable prerequisite for their successful treatment. The enormous cost to human health attributable to these disorders emphasizes the need for a more complete understanding of this area.

1. Edholm, O.G. Energy balance in man. *J. Human Nutr.* **31,** 413-431 (1977). [Context Link]

2. Montague, C. *et al.* Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* **387,** 903-908 (1997). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

3. Clement, K. *et al.* A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* **392,** 330-331 (1998). [Context Link]

4. Vaisse, C., Clement, K., Guy-Grand, B. & Froguel, P. A frameshift mutation in human MC4R is associated with a dominant form of obesity. *Nature Genet.* **20,** 113-114 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

5. Yeo, G. *et al.* A frameshift mutation in MC4R associated with dominantly inherited human obesity. *Nature Genet.* **20,** 111-112 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

6. Harris, R. B. S., Kasser, T. R. & Martin, R. J. Dynamics of recovery of body composition after overfeeding, food restriction or starvation of mature female rats. *J. Nutr.* **116,** 2536-2546 (1986). [Medline Link] [BIOSIS Previews Link] [Context Link]

7. Kennedy, G. C. The role of depot fat in the hypothalamic control of food intake in the rat. *Proc. R. Soc. Lond. B* **140,** 579-592 (1953). [Context Link]

8. Gibbs, J., Young, R. C. & Smith, G. P. Cholecystokinin decreases food intake in rats. *J. Comp. Physiol. Psychol.* **84,** 488-495 (1973). [Context Link]

9. Baura, G. *et al.* Saturable transport of insulin from plasma into the central nervous system of dogs in vivo: a mechanism for regulated insulin delivery to the brain. *J. Clin. Invest.* **92,** 1824-1830 (1993). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

10. Woods, S., Lotter, E., McKay, L. & Porte, D. J. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature* **282,** 503-505 (1979). [Medline Link] [BIOSIS Previews Link] [Context Link]

11. Zhang, Y. *et al.* Positional cloning of the mouse obese gene and its human homologue. *Nature* **372,** 425-432 (1994). [Medline Link] [BIOSIS Previews Link] [Context Link]

12. Bagdade, J. D., Bierman, E. L. & Porte, D. Jr The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. *J. Clin. Invest.* **46,** 1549-1557 (1967). [Medline Link] [Context Link]

13. Considine, R. V. *et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *New Eng. J. Med.* **334,** 292-295 (1996). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

14. Schwartz, M. W. *et al.* Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nature Med.* **2,** 589-593 (1996). [Medline Link] [BIOSIS Previews Link] [Context Link]

15. Baskin, D. G., Wilcox, B. J., Figlewicz, D. P. & Dorsa, D. M. Insulin and insulin-like growth factors in the CNS. *Trends Neurosci.* **11,** 107-111 (1988). [Medline Link] [BIOSIS Previews Link] [Context Link]

16. Baskin, D., Breininger, J. & Schwartz, M. Leptin receptor mRNA identifies a subpopulation of neuropeptide Y neurons activated by fasting in rat hypothalamus. *Diabetes* **48,** 828-833 (1999). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

17. Cheung, C., Clifton, D. & Steiner, R. Proopimelanocortin neurons are direct targets for leptin in the hypothalamus. *Endocrinology* **138,** 4489-4492 (1997). [Medline Link] [Context Link]

18. Weigle, D. S. *et al.* Recombinant *ob* protein reduces feeding and body weight in the *ob/ob* mouse. *J. Clin. Invest.* **96,** 2065-2070 (1995). **[Fulltext Link]** [Medline Link] [Context Link]

19. Campfield, L., Smith, F., Gulsez, Y., Devos, R. & Burn, P. Mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* **269,** 546-549 (1995). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

20. Sipols, A. J., Baskin, D. G. & Schwartz, M. W. Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. *Diabetes* **44,** 147-151 (1995). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

21. Porte, D. J., Woods, S., Baskin, D., Seeley, R. & Schwartz, M. Obesity, diabetes and the central nervous system. *Diabetologia* **41,** 863-881 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

22. Schwartz, M. W. *et al.* Evidence that plasma leptin and insulin levels are associated with body adiposity via different mechanisms. *Diabetes Care* **20,** 1476-1481 (1997). **[Fulltext Link]** [Medline Link] [Context Link]

23. Kahn, S. E. *et al.* Quantification of the relationship between insulin sensitivity and B-cell function in human subjects: evidence for a hyperbolic function. *Diabetes* **42,** 1663-1672 (1993). [Medline Link] [BIOSIS Previews Link] [Context Link]

24. Polonsky, K. S., Given, B. D. & VanCauter, E. Twenty-four hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. *J. Clin. Invest.* **81,** 442-448 (1988). [Medline Link] [BIOSIS Previews Link] [Context Link]

25. Mueller, W. *et al.* Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. *Endocrinology* **139,** 551-558 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

26. Wang, J., Liu, R., Hawkins, M., Barzilai, N. & Rossetti, L. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. *Nature* **393,** 684-688 (1998). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

27. Leedom, L. J. & Meehan, W. P. The psychoneuroendocrinology of diabetes mellitus in rodents. *Psychoneuroendocrinology* **14,** 275-294 (1989). [Medline Link] [BIOSIS Previews Link] [Context Link]

28. Havel, P. *et al.* Marked and rapid decreases of circulating leptin in streptozotocin diabetic rats: reversal by insulin. *Am. J. Physiol.* **274,** R1482-R1491 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

29. Hathout, E. *et al.* Changes in plasma leptin during the treatment of diabetic ketoacidosis. *J. Clin. Endocrinol. Metab.* **84,** 4545-4548 (1999). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

30. Sindelar, D. *et al.* Low plasma leptin levels contribute to diabetic hyperphagia in rats. *Diabetes* **48,** 1275-1280 (1999). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

31. Chua, S. C. *et al.* Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (Leptin) receptor. *Science* **271,** 994-996 (1996). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

32. Mantzoros, C. *et al.* Severe leptin resistance in brown fat-deficient uncoupling protein promoter-driven diphtheria toxin A mice despite suppression of hypothalamic neuropeptide Y and circulating corticosterone. *Diabetes* **47,** 230-238 (1998). [Context Link]

33. Marsh, D. J. *et al.* Response of melanocortin-4 receptor-deficient mice to anorectic and orexigenic peptides. *Nature Genet* 21, 119-122 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

34. Wilson, B. *et al.* Physiological and anatomical circuitry between Agouti-related protein and leptin signaling. *Endocrinology* **140,** 2387-2397 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

35. Bjorbaek, C. *et al.* Expression of leptin receptor isoforms in rat brain microvessels. *Endocrinology* **139,** 3485-3491 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

36. Caro, J. F. *et al.* Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* **348,** 159-161 (1996). [Medline Link] [Context Link]

37. Bjorbaek, C., Elmquist, J., Frantz, J., Shoelson, S. & Flier, J. Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol. Cell* **1,** 619-625 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

38. Stanley, B. G., Kyrkouli, S. E., Lampert, S. & Leibowitz, S. F. Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. *Peptides* **7,** 1189-1192 (1986). [Medline Link] [BIOSIS Previews Link] [Context Link]

39. Billington, C. J., Briggs, J. E., Grace, M. & Levine, A. S. Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. *Am. J. Physiol.* **260,** R321-R327 (1991). [Medline Link] [BIOSIS Previews Link] [Context Link]

40. Zarjevski, N., Cusin, I., Vetter, R., Rohner-Jeanrenaud, F. & Jeanrenaud, B. Chronic intracerebroventricular neuropeptide-Y administration to normal rats mimics hormonal and metabolic changes of obesity. *Endocrinology* **133,** 1753-1758 (1993). [Medline Link] [BIOSIS Previews Link] [Context Link]

41. Kalra, S. P., Dube, M. G., Sahu, A., Phelps, C. P. & Kalra, P. Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. *Proc. Natl Acad. Sci. USA* **88,** 10931-10935 (1991). [Medline Link] [BIOSIS Previews Link] [Context Link]

42. White, J. D. & Kershaw, M. Increased hypothalamic neuropeptide Y expression following food deprivation. *Mol. Cell. Neurosci.* **1,** 41-48 (1989). [BIOSIS Previews Link] [Context Link]

43. Wilding, J. P. H. *et al.* Increased neuropeptide-Y messenger ribonucleic acid (mRNA) and decreased neurotensin mRNA in the hypothalamus of the obese (*ob/ob*) mouse. *Endocrinology* **132,** 1939-1944 (1993). [Medline Link] [BIOSIS Previews Link] [Context Link]

44. Schwartz, M. W. *et al.* Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in *ob/ob* mice. *Diabetes* **45,** 531-535 (1996). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

45. Stephens, T. W. *et al.* The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* **377,** 530-534 (1995). **[Fulltext Link]** [Medline Link] [Context Link]

46. Erickson, J. C., Hollopeter, G. & Palmiter, R. D. Attenuation of the obesity syndrome of *ob/ob* mice by the loss of neuropeptide Y. *Science* **274,** 1704-1707 (1996). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

47. Williams, G. *et al.* Increased neuropeptide Y concentrations in specific hypothalamic regions of streptozocin-induced diabetic rats. *Diabetes* **38,** 321-327 (1989). [Medline Link] [BIOSIS Previews Link] [Context Link]

48. Erickson, J. C., Clegg, K. E. & Palmiter, R. D. Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature* **381,** 415-418 (1996). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

49. Cone, R. *et al.* The melanocortin receptors: agonists, antagonists, and the hormonal control of pigmentation. *Rec. Prog. Horm. Res.* **51,** 287-318 (1996). [Medline Link] [BIOSIS Previews Link] [Context Link]

50. Bray, G. A., Fisler, J. & York, D. A. Neuroendocrine control of the development of obesity: understanding gained from studies of experimental animal models. *Front. Neuroendocrinol.* **11,** 128-181 (1990). [Context Link]

51. Kow, L. & Pfaff, D. The effects of the TRH-metabolite cyclo (His-Pro) and its analogs on feeding. *Pharmacol. Biochem. Behav.* **38,** 359-364 (1991). [Medline Link] [BIOSIS Previews Link] [Context Link]

52. Kristensen, P. *et al.* Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* **393,** 72-76 (1998). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

53. Luheshi, G., Gardner, J., Rushforth, D., Loudon, A. & Rothwell, N. Leptin actions on food intake and body temperature are mediated by IL-1. *Proc. Natl Acad. Sci. USA* **96,** 7047-7052 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

54. Mountjoy, K., Mortrud, M., Low, M., Simerly, R. & Cone, R. Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. *Mol. Endocrinol.* **8,** 1298-1308 (1994). [Medline Link] [BIOSIS Previews Link] [Context Link]

55. Fan, W., Boston, B., Kesterson, R., Hruby, V. & Cone, R. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* **385,** 165-168 (1997). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

56. Huszar, D. *et al.* Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* **88,** 131-141 (1997). [Medline Link] [BIOSIS Previews Link] [Context Link]

57. Miller, M. *et al.* Cloning of the mouse agouti gene predicts a secreted protein ubiquitously expressed in mice carrying the lethal yellow mutation. *Genes Dev.* **7,** 454-467 (1993). [Medline Link] [BIOSIS Previews Link] [Context Link]

58. Shutter, J. *et al.* Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. *Genes Dev.* **11,** 593-602 (1997). [Medline Link] [BIOSIS Previews Link] [Context Link]

59. Ollmann, M. *et al.* Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science* **278,** 135-138 (1997). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

60. Hahn, T., Breininger, J., Baskin, D. & Schwartz, M. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nature Neurosci* **1,** 271-272 (1998). [Context Link]

61. Broberger, C., Johansen, J., Johasson, C., Schalling, M. & Hokfelt, T. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proc. Natl Acad. Sci. USA* **95,** 15043-15048 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

62. Hagan, M. *et al.* Effect of agouti-related peptide (83-132) on food intake: competitive melanocortin receptor antagonism on short but not long term effects. *Am. J. Physiol.* (in the press). [Context Link]

63. Rossi, M. *et al.* A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. *Endocrinology* **139,** 4428-4431 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

64. Stellar, E. The physiology of motivation. *Psychol. Rev.* **61,** 5 (1954). [Context Link]

65. Woods, S., Seeley, R., Porte, D. J. & Schwartz, M. Signals that regulate food intake and energy homeostasis. *Science* **280,** 1378-1383 (1998). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

66. Elias, C. *et al.* Leptin activates hypothalamic CART neurons projecting to the spinal cord. *Neuron* **21,** 1375-1385 (1998). [Context Link]

67. Satoh, N. *et al.* The arcuate nucleus as a primary site of satiety effect of leptin in rats. *Neurosci. Lett.* **224,** 149-152 (1997). [Medline Link] [BIOSIS Previews Link] [Context Link]

68. Tang-Christensen, M., Holst, J., Hartmann, B. & Vrang, N. The arcuate nucleus is pivotal in mediating the anorectic effects of centrally administered leptin. *Neuroreport* **10,** 1183-1187 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

69. Dawson, R., Pelleymounter, M., Millard, W., Liu, S. & Eppler, B. Attenuation of leptin-mediated effects by monosodium glutamate-induced arcuate nucleus damage. *Am. J. Physiol.* **273,** E202-E206 (1997). [Medline Link] [BIOSIS Previews Link] [Context Link]

70. Schwartz, M. *et al.* Leptin increases hypothalamic proopiomelanocortin (POMC) mRNA expression in the rostral arcuate nucleus. *Diabetes* **46,** 2119-2123 (1997). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

71. Thornton, J., Cheung, C., Clifton, D. & Steiner, R. Regulation of hypothalamic proopiomelanocortin mRNA by leptin in *ob/ob* mice. *Endocrinology* **138,** 5063-5066 (1997). [Medline Link] [BIOSIS Previews Link] [Context Link]

72. Hagan, M. *et al.* Role of the CNS melanocortin system in the response to overfeeding. *J. Neurosci.* **19,** 2362-2367 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

73. Seeley, R. *et al.* Melanocortin receptors in leptin effects. *Nature* **390,** 349 (1997). **[Fulltext Link]** [Medline Link]

[Context Link]

74. Elmquist, J., Maratos-Flier, E., Saper, C. & Flier, J. Unraveling the central nervous system pathways underlying responses to leptin. *Nature Neurosci.* 1, 445-450 (1998). [Context Link]

75. Elmquist, J., Elias, C. & Saper, C. From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron* **22,** 221-232 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

76. Stanley, B. G., Willett, V. L. III, Donias, H. W., Ha, L. H. & Spears, L. C. The lateral hypothalamus: a primary site mediating excitatory amino acid-elicited eating. *Brain Res.* **630,** 41-49 (1993). [Medline Link] [BIOSIS Previews Link] [Context Link]

77. Dallman, M. F. *et al.* Feast and famine: Critical role of glucocorticoids with insulin in daily energy flow. *Front. Neuroendocrinol.* **14,** 303-347 (1993). [Medline Link] [BIOSIS Previews Link] [Context Link]

78. Verbalis, J., Blackburn, R., Hoffman, G. & Stricker, E. Establishing behavioral and physiological functions of central oxytocin: insights from studies of oxytocin and ingestive behaviors. *Adv. Exp. Med. Biol.* **395,** 209-225 (1995). [Medline Link] [Context Link]

79. Qu, D. *et al.* A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature* **380,** 243-247 (1996). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

80. Shimada, M., Tritos, N., Lowell, B., Flier, J. & Maratos-Flier, E. Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature* **39,** 670-674 (1998). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

81. Saito, Y. *et al.* Molecular characterization of the melanin-concentrating-hormone receptor. *Nature* **400,** 265-269 (1999). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

82. Chambers, J. *et al.* Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1. *Nature* **400,** 261-265 (1999). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

83. de Lecea, L. *et al.* The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc. Natl Acad. Sci. USA* **95,** 322-327 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

84. Sakurai, T. *et al.* Orexins and orexin receptors: a family of hypothalamic neuropeptides and G-protein-coupled receptors that regulate feeding behavior. *Cell* **92,** 573-585 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

85. Hagan, J. *et al.* Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc. Natl Acad. Sci. USA* **96,** 10911-10916 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

86. Chemelli, R. *et al.* Narcolepsy in *orexin* knockout mice: molecular genetics of sleep regulation. *Cell* **98,** 437-451 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

87. Figlewicz, D. P., Stein, L. J., West, D., Porte, D. Jr & Woods, S. C. Intracisternal insulin alters sensitivity to CCK-induced meal suppression in baboons. *Am. J. Physiol.* **250,** R856-R860 (1986). [Medline Link] [BIOSIS Previews Link] [Context Link]

88. Woods, S. C. & Strubbe, J. H. The psychobiology of meals. *Psychonomic Bull. Rev.* 1, 141-155 (1994). [Context Link]

89. Leibowitz, S. & Alexander, J. Analysis of neuropeptide Y-induced feeding: dissociation of Y1 and Y2 receptor effects on natural meal patterns. *Peptides* **12,** 1251-1260 (1991). [Medline Link] [BIOSIS Previews Link] [Context Link]

90. Flynn, M., Scott, T., Pritchard, T. & Plata-Salaman. C. Mode of action of OB protein (leptin) on feeding. *Am. J. Physiol.* **275,** R174-R179 (1998). [Context Link]

91. Ritter, S., Dinh, T. & Friedman, M. Induction of Fos-like immunoreactivity (Fos-li) and stimulation of feeding by 2,5-anhydro-D-mannitol (2,5-AM) require the vagus nerve. *Brain Res.* **646,** 53-64 (1994). [Medline Link] [BIOSIS Previews Link] [Context Link]

92. Travers, S. & Norgren, R. Gustatory neural processing in the hindbrain. *Annu. Rev. Neurosci.* **10,** 595-632 (1987). [Medline Link] [BIOSIS Previews Link] [Context Link]

93. Friedman, M., Harris, R., Ji, H., Ramirez, I. & Tordoff, M. Fatty acid oxidation affects food intake by altering hepatic energy status. *Am. J. Physiol.* **276,** R1046-R1053 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

94. Moran, T. & Schwartz, G. Neurobiology of cholecystokinin. *Crit. Rev. Neurobiol.* **9,** 1-28 (1994). [Context Link]

95. Grill, H. & Smith, G. Cholecystokinin decreases sucrose intake in chronic decrebrate rats. *Am. J. Physiol.* **254,** R853-R856 (1988). [Medline Link] [BIOSIS Previews Link] [Context Link]

96. Matson, C. & Ritter, R. Long-term CCK-leptin synergy suggests a role for CCK in the regulation of body weight. *Am. J. Physiol.* **276,** R1038-R1045 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

97. Emond, M., Schwartz, G., Ladenheim, E. & Moran, T. Central leptin modulates behavioral and neural responsivity to CCK. *Am. J. Physiol.* **276,** R1545-R1549 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

98. Ter Horst, G., de Boer, P., Luiten, P. & van Willigen, J. Ascending projections from the solitary tract nucleus to the hypothalamus. A *Phaseolus vulgaris* lectin tracing study in the rat. *Neuroscience* **31,** 785-797 (1989). [Medline Link] [BIOSIS Previews Link] [Context Link]

99. Grill, H., Ginsberg, A., Seeley, R. & Kaplan, J. Brainstem application of melanocortin receptor ligands produces long-lasting effects on feeding and body weight. *J. Neurosci.* **18,** 10128-10135 (1998). [Medline Link] [Context Link]

100. Mercer, I., Moar, K. & Hoggard, N. Localization of leptin receptor (Ob-R) messenger ribonucleic acid in the rodent hindbrain. *Endocrinology* **139,** 29-34 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

101. Bronstein, D., Schafer, M., Watson, S. & Akil, H. Evidence that beta-endorphin is synthesized in cells in the nucleus tractus solitarius: detection of POMC mRNA. *Brain Res.* **587,** 269-275 (1992). [Medline Link] [BIOSIS Previews Link] [Context Link]

102. Leibowitz, S., Roossin, P. & Rosenn, M. Chronic norepinephrine injection into the hypothalamic paraventricular nucleus produces hyperphagia and increased body weight in the rat. *Pharmacol. Biochem. Behav.* **21,** 801-808 (1984). [Medline Link] [BIOSIS Previews Link] [Context Link]

103. Oltmans, G. Norepinephrine and dopamine levels in hypothalamic nuclei of the genetically obese mouse (*ob/ob*). *Brain Res.* **273,** 369-373 (1983). [Medline Link] [BIOSIS Previews Link] [Context Link]

104. Brunetti, L., Michelotto, B., Orlando, G. & Vacca, M. Leptin inhibits norepinephrine and dopamine release from rat hypothalamic neuronal endings. *Eur. J. Pharmacol.* **372,** 237-240 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

105. Salamone, I., Mahan, K. & Rogers, S. Ventrolateral striatal dopamine depletions impair feeding and food handling in rats. *Pharmacol. Biochem. Behav.* **44,** 605-610 (1993). [Medline Link] [BIOSIS Previews Link] [Context Link]

106. Szczypka, M. *et al.* Feeding behavior in dopamine-deficient mice. *Proc. Natl Acad. Sci. USA* **96,** 12138-12143 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

107. Pothos, E., Creese, I. & Hoebel, B. Restricted eating with weight loss selectively decreases extracellular dopamine in the nucleus accumbens and alters dopamine response to amphetamine, morphine, and food intake. *J. Neurosci.* **15,** 6640-6650 (1995). [Medline Link] [BIOSIS Previews Link] [Context Link]

108. Figlewicz, D., Endocrine regulation of neurotransmitter transporters. *Epilepsy Res.* **37,** 203-210 (1999). [Medline Link] [BIOSIS Previews Link] [Context Link]

109. Patterson, T. *et al.* Food deprivation decreases mRNA and activity of the rat dopamine transporter. *Neuroendocrinology* **68,** 11-20 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

110. Leibowitz, S. & Alexander, J. Hypothalamic serotonin in control of eating behavior, meal size, and body weight. *Biol. Psychiatry* **44,** 851-864 (1998). [Medline Link] [BIOSIS Previews Link] [Context Link]

111. Nonogaki, K., Strack, A., Dallman, M. & Tecott, L. Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT_{2C} receptor gene. *Nature Med.* **4,** 1152-1156 (1998). [Medline Link] [Context Link]

112. Calapai, G. *et al.* Leptin increases serotonin turnover by inhibition of brain nitric oxide synthesis. *J. Clin.*

Invest. **104,** 975-982 (1999). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

113. Yaswen, L., Diehl, N., Brennam, M. & Hochgeschwender, V. Obesity in the mouse model of proopiomelanocortin deficiency responds to peripheral melanocortin. *Nature Med.* **5,** 1066-1070 (1999). [Medline [BIOSIS Previews Link] [Context Link]

114. Farooqi, I. *et al.* Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N. Engl. J. Med.* **341,** 879-884 (1999). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link] [Context Link]

115. Cheatham, B. & Kahn, C. Insulin action and the insulin signaling network. *Endocr. Rev.* **16,** 117-142 (1995). [Medline Link] [BIOSIS Previews Link]

116. Baskin, D. G., Sipols, A. J., Schwartz, M. W. & White, M. F. Insulin receptor substrate-1 (IRS-1) expression in rat brain. *Endocrinology* **134,** 1952-1955 (1994). [Medline Link] [BIOSIS Previews Link]

117. Kapeller, R. *et al.* Tyrosine phosphorylation of tub and its association with Src homology 2 domain-containing proteins implicate tub in intracellular signaling by insulin. *J. Biol. Chem.* **274,** 24980-24986 (1999). [Medline Link] [BIOSIS Previews Link]

118. Tartaglia, L. A. *et al.* Identification and expression cloning of a leptin receptor, OB-R. *Cell* **83,** 1263-1271 (1995). [Medline Link] [BIOSIS Previews Link]

119. Tartaglia, L. A. The leptin receptor. *J. Biol. Chem.* 272, 6093-6096 (1997). [Medline Link] [BIOSIS Previews Link]

120. Spanswick, D., Smith, M., Groppi, V., Logam, S. & Ashford, M. Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature* **390,** 521-525 (1997). **[Fulltext Link]** [Medline Link] [BIOSIS Previews Link]

121. Dunn-Meynell, A., Rawson, N. & Levin, B. Distribution and phenotype of neurons containing the ATP-sensitive K^+ channel in rat brain. *Brain Res.* **814,** 41-54 (1998). [Medline Link] [BIOSIS Previews Link]

Acknowledgements

Assistance in manuscript preparation provided by L. Walters and in figure preparation by M. Baskin is gratefully acknowledged. This work was supported by NIH grants, the Diabetes Endocrinology Research Center and Clinical Nutrition Research Unit of the University of Washington, and the Department of Veterans Affairs.

Accession Number: 00006056-200004060-00042
