Hunger and Satiety: A View From the Brain

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The regulation of energy homeostasis is an area that straddles neurobiology, classical endocrinology, and metabolism. It is currently one of the most exciting and rapidly advancing topics in biomedical research; it is also one of the most frustrating, because the numerous leaps in scientific understanding have not yet been rewarded by any major breakthrough in the practical treatment of human nutritional disorders. Foremost among these is obesity, which is fast tightening its grip on mankind and is threatening to become one of the greatest threats to global health of the new millennium.

Here, we shall review some recent progress in understanding how the body’s energy stores and nutritional status are able to signal their presence to the brain, and the mechanisms through which the brain senses and responds to these signals. Much of this work has, necessarily, been performed in rodents, but some of the lessons learned in lower mammals may also apply to man—although the strength and emphasis of the message may differ between the species.

We shall begin by describing the basic structure of the hypothalamus, the main area involved in energy homeostasis in both rodents and man. This provides an anatomic framework for the neuronal populations, identified by the specific neurotransmitters which they express, which are important in sensing and integrating nutritional signals and in translating these inputs into appropriate changes in feeding behavior and energy expenditure. At the end of the review, we shall discuss some possible therapeutic avenues that may be opened up by this research.

NEUROANATOMY OF ENERGY HOMEOSTASIS

The basic organization of the rat hypothalamus is illustrated in Fig. 1. The arcuate nucleus (ARC) lies in the floor of the third ventricle immediately above the median eminence (ME), and occupies almost half the length of the hypothalamus. The ARC contains several functionally discrete populations of neurons, including one that expresses the orexigenic (appetite-stimulating) neuropeptides, neuropeptide Y (NPY) and agouti-related peptide (AGRP), and another that contains pro-opiomelanocortin (POMC) and cocaine- and amphetamine-related transcript (CART), which
both act to inhibit food intake (1). The ARC and ME lie within the mediobasal hypothalamus, where the blood-brain barrier (BBB) is specially modified to render this region readily accessible to circulating hormones such as leptin, insulin, ghrelin, and the glucocorticoids. As will be discussed, these are all involved in signaling nutritional state and in regulating appetite. The ARC has extensive reciprocal connections with other hypothalamic regions that control energy balance, including the paraventricular nuclei (PVN), ventromedial hypothalamic (VMH) and dorsomedial hypothalamic (DMH) nuclei; and the lateral hypothalamic area (LHA).
The PVN, located beside the top of the third ventricle in the anterior hypothalamus, is the site of convergence for many neuronal pathways that regulate energy homeostasis; these include projections from the NPY/AGRP and POMC/CART neurons in the ARC, and from the orexin neurons of the LHA. The PVN also contains neurons that express corticotropin-releasing factor, an appetite-inhibiting peptide that is released in the ARC and apparently acts to inhibit the NPY neurons. Neuronal pathways project from the PVN to the vagal nuclei of the medulla, which in turn innervate the islets of Langerhans; injection of various neurotransmitters into the PVN can modulate insulin and glucagon secretion (2). Lesions of the PVN result in hyperphagia, reduced energy expenditure, and obesity (3).

The large VMH was long considered to be a "satiety center", because stimulation of the nucleus inhibits feeding, while lesions in the region result in hyperphagia and weight gain (4). Although this hypothesis now appears to be over-simplistic, recent studies have shown that the long isoform of the leptin receptor (Ob-Rb) is highly abundant in VMH neurons (5), suggesting that this region is an important target for circulating leptin, the adipocyte-derived hormone, which acts on the brain to inhibit feeding. The VMH has direct connections with the PVN and DMH and through them, may connect indirectly with the LHA.

The diffuse LHA contains separate populations of neurons that express the orexigenic peptides, Orexin A and melanin-concentrating hormone (MCH). NPY terminals are abundant in the LHA where they form synaptic connections with the Orexin and MCH cell bodies, and this region is rich in the NPY Y5 receptors that are proposed to mediate the appetite-stimulating effects of NPY (6). The LHA was viewed classically as a 'feeding center', whose actions oppose those of the VMH: electrical stimulation of this region increases food intake, while damage here can lead to fatal anorexia and wasting. This area is also rich in glucose-sensitive neurons (GSN) that are excited by a decrease in blood glucose concentration, which is a powerful stimulus to feeding. The apparently reciprocal activities of the LHA and VMH are reiterated in the context of glucose-sensing: the VMH is rich in glucose-responsive neurons (GRN), which respond to an increase in blood glucose levels and may help to terminate feeding (7).

The DMH, located immediately above the VMH, contains abundant insulin and leptin (Ob-Rb) receptors. ARC NPY/AGRP neurons also terminate here. The DMH has extensive connections with other hypothalamic nuclei; it and the PVN are thought to integrate opposing signals from the VMH and LHA, effectively acting as a functional unit that initiates, maintains, and ultimately terminates feeding (8).

The structure of the human hypothalamus is broadly similar to that in the rat. Although details of the neuronal circuitry have not been reported, the distributions of major neuropeptides such as NPY, POMC, and Orexin A appear to conform with those in rodents.

MEDULLARY AREAS

The regulation of energy homeostasis also involves multiple brain regions outside the hypothalamus. The medulla contains the nucleus tractus solitarius (NTS), which
receives sensory inputs from the viscera and relays these to the hypothalamus. These visceral signals carried by sensory (afferent) fibers of the vagus nerve include gastric distension and portal-vein glucose levels, and thus describe nutritional state as viewed by the gut. The intestinal peptide cholecystokinin (CCK), which is released by the gut and is involved in meal termination, also signals to the NTS via receptors (CCKA) on sensory terminals of the vagal nerve. Taste is another sensory modality conveyed to the NTS.

Some NTS neurons are glucose-receptive; others express POMC and the melanocortin-4 receptors (MC4-R) that are the target for the POMC product, α-melanocyste-stimulating hormone (α-MSH); administration of MC4-R agonists and antagonists into the fourth ventricle (adjacent to the NTS) affects feeding responses in the same way as when injected into the hypothalamus. Leptin receptors are also expressed, and the NTS—like the ARC and ME—lies 'outside' the BBB and is accessible to circulating signaling molecules such as leptin. Destruction of the NTS leads to over-consumption of palatable food, confirming that the NTS and hypothalamus both play important roles in energy homeostasis.

HYPOTHALAMIC PATHWAYS AND NEUROTRANSMITTERS

The hypothalamus contains a wealth of neurotransmitters and peptides—to date, over 50 have been reported. They include the monoamines (serotonin, noradrenaline, adrenaline, dopamine), acetylcholine, and other classical neurotransmitters, together with an ever-expanding list of peptides. Many can influence feeding behavior and energy metabolism under experimental conditions in rodents. Some of the more convincing candidates will be discussed.

Most of these neurotransmitters have been identified within the human hypothalamus, but relatively little is known of their neuronal pathways, sites of release, or possible functions in humans.

Neuropeptide Y

NPY, a 36-amino acid neurotransmitter belonging to the pancreatic polypeptide family, is one of the most abundant and widely distributed neurotransmitters in the mammalian central nervous system, including that of man. NPY concentrations are particularly high in the hypothalamus, mostly derived from neurons in the ARC (Fig. 1), 90% of which also express AGRP (9). The bulk of ARC NPY neurons project to the PVN and DMH, which also receive inputs from NPY-containing fibers ascending from adrenergic nuclei in the medulla. There are also short projections that terminate within the ARC itself; NPY is thought to inhibit POMC neurons (via Y1 receptors) and may also inhibit the NPY neurons themselves via Y2 receptors (10) (Fig. 2).

NPY injected into cerebral ventricles or directly into the PVN, DMH, or LHA, induces pronounced hyperphagia—indeed, NPY is one of the most potent central appetite stimulants known. It also reduces thermogenesis, through inhibition of the
FIG. 2. Interactions between pro-opiomelanocortin and neuropeptide Y neuronal projections within the hypothalamus

Sympathetic outflow to brown adipose tissue and other thermogenic tissues. Chronic NPY administration induces obesity, which mimics the features of genetically obese rodents (ob/ob and db/db mice and the phenotypically similar fa/fa Zucker rat); as will be discussed, this resemblance is more than mere coincidence because overactivity of the ARC NPY neurons is thought to contribute to hyperphagia, reduced thermogenesis, and adiposity in these mutants. The orexigenic action of NPY is thought to be mediated by specific subtypes of NPY receptors, probably Y5, with Y1 likely to play an additional role (11).

ARC NPY neurons become overactive in animals that have lost body weight and fat through energy deficits, such as in starvation, lactation, or insulin-deficient diabetes. NPY expression is increased, and elevated NPY release has been confirmed directly by stereotactic sampling in the PVN of starved and diabetic animals (12). The NPY neurons may be stimulated under these circumstances by decreases in circulating levels of insulin or leptin, which both inhibit NPY gene expression in the ARC (13), or by the increase in corticosterone concentration, which is stimulatory; the ARC NPY neurons express both Ob-Rb leptin receptors and glucocorticoid
receptors. ARC NPY neurons are also overactive in rodents with genetic obesity that is due either to leptin receptor defects (e.g., caused by db/db and fa/fa mutations in the mouse and rat, respectively) or to loss of biologically active leptin (ob/ob mouse). This disinhibition implies that leptin is normally a crucial regulator of the ARC NPY neurons. However, NPY does not appear to mediate overeating under all conditions, nor in all forms of obesity. ARC NPY neuronal activity is reduced in rats with dietary obesity induced by voluntary overeating of a palatable diet; the neurons may be inhibited in an attempt to limit overeating and weight gain, possibly in response to the increase in plasma leptin that occurs during the development of dietary obesity (14).

Surprisingly, transgenic 'knockout' mice that lack NPY eat and grow normally (15). In our view, this does not rule out a role for the NPY system in regulating food intake, but instead highlights the potential for other neuronal systems or transmitters to take over from NPY—a general caveat when using the knockout approach to explore the control of energy homeostasis. It has recently been shown that AGRP messenger mRNA and immunoreactivity are upregulated with fasting in NPY knockout mice, suggesting that AGRP (also produced by the NPY neurons) may compensate for the lack of NPY in this model (16).

Overall, the ARC NPY neurons appear to serve a protective, anti-starvation function, acting to correct the effects of prolonged periods of inadequate food supply by increasing the drive to locate and consume food, while limiting energy expenditure to prevent further depletion of body energy stores.

Melanocyte-Stimulating Hormones

The melanocortin neurons produce various peptides derived from a common precursor, POMC. Of these, α-melanocyte-stimulating hormone (α-MSH) inhibits feeding when injected centrally and is considered to be the most important melanocortin regulator of feeding; β-MSH may also play a similar role. POMC is synthesized in specific neurons of the ARC and NTS, and discrete α-MSH-containing pathways project from the ARC to many brain regions, particularly elsewhere in the hypothalamus. Three melanocortin receptor subtypes, MC3-R, MC4-R, and MC5-R, have been located in the brain; both MC3-R and MC4-R are expressed within specific hypothalamic nuclei, including the VMH, DMH, and ARC-ME (17,18).

The activity of the melanocortin system interaction can be modulated by endogenous antagonists, as well as the agonist, α-MSH. The first to be discovered was a 131-amino acid peptide termed "agouti," responsible for the striking phenotype of the obese yellow (AY) mouse, a long-recognized rodent model of obesity. The cause is a mutation in the promoter region of the agouti gene, which results in ectopic expression of agouti peptide in numerous sites, including the hypothalamus; here, antagonism of MC3-R and MC4-R leads to hyperphagia, reduced energy expenditure, and ultimately obesity (19). Thus, it was suggested that α-MSH acts tonically via the hypothalamic melanocortin receptors, to restrain food intake and body mass;
under normal physiologic conditions, this system is held in check by another melanocortin antagonist, AGRP, as will be discussed.

Both MC3-R and MC4-R probably mediate the hypophagic effects of the melanocortins, but recent studies have given MC4-R a central role. MC4-R knockout mice display obesity similar to that of the A\textsuperscript{Y} mouse (20). Additionally, powerful modulators of feeding are more selective for MC4-R than MC3-R while an MC3-R preferring agonist, $\gamma$-MSH, has little effect on food intake (21). Furthermore, MC4-R in key appetite-regulating hypothalamic nuclei have been shown to be selectively regulated in rats subjected to altered nutritional state (underfeeding or overfeeding), while the density of MC3-R does not change in these conditions (18). On the other hand, the MC3-R knockout mouse becomes obese; adiposity develops despite reduced food intake, apparently because of greater feeding efficiency, while mice lacking both MC3-R and MC4-R demonstrate greater obesity than that of MC4-R deficiency alone (22). Thus, both these melanocortin receptors probably participate in the regulation of body weight.

The melanocortin system responds to various peripheral signals of nutritional status, notably leptin. Approximately 30% of ARC POMC neurons express the Ob-Rb isoform of the leptin receptor, and intraperitoneal leptin administration increases hypothalamic POMC mRNA levels, while conditions associated with decreased leptin (e.g., fasting) or mutations that cause loss of the leptin signal (ob/ob and fa/fa/) show decreased POMC mRNA levels (23). Leptin therefore appears to stimulate POMC neurons, consistent with the observation that both inhibit feeding. There is also evidence that leptin may act on a particular subset of neurons (either POMC or NPY/AGRP) that project specifically to MC4-R in the VMH and so determine an individual rat's susceptibility to dietary-induced obesity (24); through this interaction, a relative increase in leptin soon after exposure to a palatable diet may somehow 'program' this pathway to resist overeating in the longer term.

A unique feature of the melanocortin system is the presence of the endogenous antagonist, AGRP. AGRP expression is limited to the NPY neurons of the ARC, but it is thought to be released into the same synaptic complex as $\alpha$-MSH in some hypothalamic nuclei. AGRP injected centrally (or overexpressed in transgenic mice) causes marked and prolonged hyperphagia—food intake may be stimulated for several days after a single injection—and this can override leptin-induced inhibition of feeding (25). Interestingly, AGRP may be regulated more robustly by altered metabolic status than the melanocortins themselves because changes in AGRP concentrations are observed in dietary-obese and food-restricted animals in the absence of any alterations in $\alpha$-MSH or POMC (26). This suggests that AGRP may fine-tune the activity of the melanocortin axis and thus its tonic restraining effect on food intake and body weight. Recent evidence indicates that AGRP may stimulate feeding through an additional mechanism that is independent of its antagonism of melanocortin receptors (27). AGRP expression is increased in ob/ob and db/db mice, and probably contributes to hyperphagia in these models.

The melanocortin and NPY neuronal systems of the ARC apparently interact with each other in a reciprocal fashion. MC4-R knockout mice respond to the orexigenic
effects of NPY, and the NPY Y1 selective antagonist BW1229U91 significantly attenuates the feeding effects of the MC4-R antagonist HS014 (28). MC3-R are expressed by NPY neurons and there is evidence that melanocortinergic neurons exert inhibitory control over the NPY neurons (see Fig. 2). Thus, stimulation of the ARC NPY neurons could enhance feeding through a dual action, with activation of NPY receptors and also antagonism of MC4-R by release of AGRP. Additionally, the ARC POMC neurons express Y1 receptors and receive inhibitory inputs from NPY terminals (29). Thus, NPY release could potentially inhibit the ARC melanocortin system directly at the cell body as well as postsynaptically through AGRP release.

Cocaine- and amphetamine-regulated transcript (CART) was first identified as a major brain mRNA species that was upregulated by cocaine and amphetamine (30). The CART protein is coexpressed with POMC neurons of the ARC and is an appetite-inhibiting factor that is upregulated by leptin; it may function as an anorectic neuropeptide in the regulation of food intake. However, in contrast to the marked obesity seen with knockout or mutations affecting MC4-R, POMC, or leptin, knockout of CART only predisposes mice to become obese when eating an energy-dense diet (30). Overall, CART may act synergistically with the melanocortin axis to restrain feeding.

The melanocortin axis operates in humans, as confirmed by recent observations that rare cases of morbid obesity are associated with specific mutations affecting components of the system. These include inactivation of prohormone convertase-1 (the enzyme that cleaves POMC to yield α-MSH and other melanocortins), truncation or frameshift within the POMC gene, and a frameshift mutation in the MC4-R that causes a dominant inheritance of morbid obesity (31,32). Mutations affecting this system could account for several percent of cases of morbid obesity that develop during childhood.

**Orexins**

Orexin-A and Orexin-B were discovered through their interaction with an “orphan” G-protein–coupled receptor that was isolated from hypothalamic tissue. These two homologous peptides, of 33 and 28 amino acid residues, respectively, are derived from the same precursor, prepro-Orexin, which is synonymous with preprohypocretin (33). Orexins/hypocretins are expressed by specific neurons restricted to the perifornical nuclei and dorsal and lateral areas of the hypothalamus that lie close to, but are distinct from, neurons that express MCH. Despite their circumscribed origin, Orexin neurons project extensively to many sites including the PVN, ARC, extrahypothalamic areas involved in appetite regulation (NTS and vagal nuclei), and the locus ceruleus, an important center in controlling the sleep-wake cycle (34). Orexin neurons also interact with other appetite-regulating neuronal systems, and reciprocal connections have been identified between Orexin neurons and ARC NPY/AGRP and POMC/CART populations (35). The two Orexin receptors, OX1- and OX2-R, are also widely distributed throughout the brain: OX1-R, selective for Orexin-A, is expressed abundantly in the VMH, while OX2-R, which has comparable affinity for both Orexin peptides, is found predominantly in the PVN (33).
Central Orexin administration stimulates feeding, and this action gave the peptides their name (orexis: Greek for 'appetite'). Orexin-A induces acute hyperphagia, particularly during the daytime, which is inhibited by blocking Orexin-A (with anti-Orexin-A antibodies) or OX1-R (with specific antagonists) (36,37). The stimulation of feeding is short lived: overall 24-hour intake is not increased after a single injection, and obesity does not result from chronic intracerebroventricular administration (38). Orexin-B has a weaker effect (if any) in stimulating feeding, although its levels appear to be selectively altered under conditions of increased hunger; it has been suggested that these changes in Orexin-B may be related to changes in arousal rather than hunger per se.

Most Orexin neurons express the Ob-Rb isoform of the leptin receptors, and central leptin administration has been reported to decrease Orexin expression (39), suggesting that Orexin neurons may be regulated by the status of peripheral fat stores. However, Orexin expression is not altered when plasma leptin levels decrease markedly in either food restriction or diabetes (40). It appears that nutritional conditions under which Orexin neurons are activated are very tightly defined, with decreased plasma glucose and the absence of food from the gut both being required. Acute hypoglycemia activates Orexin neurons, but this response is prevented by allowing the animals to eat, suggesting that the presence of food in the gut may generate inhibitory signals, e.g., gastric distension (40). Such signals are known to be transmitted via vagal sensory fibers to the NTS, from where an important projection ascends to the LHA in which the Orexin neuron cell bodies are situated (Fig. 3).

Current evidence indicates that the Orexins are involved in the short-term regulation of feeding rather than the long-term control of body weight. They may help to initiate feeding in response to decreases in glucose concentration, and may cooperate with GSNs in the hypothalamus to detect this signal (see following paragraphs). It is not known whether the Orexin neurons respond only to large decreases in glucose concentration or are also able to sense the small glucose decrements that precede spontaneous eating episodes in freely feeding rats and that are thought to trigger food ingestion.

The widespread distribution of the Orexin terminals and receptors indicate that these peptides may have broader functions than energy homeostasis. The Orexin system is known to be essential in regulating sleep-wake functions and is probably also involved in cardiovascular regulation of blood pressure and neuroendocrine and autonomic functions (41). Orexins may therefore mediate enhanced alertness and increased food-seeking behavior, which are among the many adaptive responses to severe energy deficit.

Melanin-Concentrating Hormone

MCH is a cyclic 19-amino acid peptide expressed predominantly in specific neurons of the LHA and the adjacent zona incerta that project extensively throughout the brain and posterior pituitary gland (42). MCH was originally named for its ability to lighten fish skin by causing melanin (in melanophores) to aggregate, but a role
FIG. 3. Possible organization of Orexin and glucose-sensitive neurons (GSN), which sense glucose changes and food in the gut. Signals from falling glucose and an empty gut converge via vagus on the nucleus tractus solitarius, which sends integrated information to the lateral hypothalamic area and activates Orexin neurons directly or indirectly through GSN in the hypothalamus. ○, O; ●, 1 Orexin neurons.

in feeding behavior and energy homeostasis is now firmly established. MCH expression is enhanced by fasting in both normal and ob/ob mice (43), and is upregulated in the hypothalamus of ob/ob mice and fa/fa Zucker rats (43). Intracerebroventricular injection of MCH significantly increases feeding in rats and can antagonize the hypophagic effect of α-MSH (44), while MCH-knockout mice show hypophagia, increased metabolic rate, and reduced fat mass (45).

The G-protein–coupled receptor MCH-1R (originally designated SLC-1), was first identified as a receptor for MCH. MCH-1R mRNA and receptor protein are distributed throughout several brain regions, with particularly high levels in the VMH and DMH (46). Expression of MCH-1R mRNA is increased in fasting and ob/ob mice, but unchanged in MCH knockout animals, suggesting that (unlike several other G-protein–coupled receptors) this receptor is regulated by levels of leptin rather than availability of the ligand itself (47). Recently, a second high-affinity MCH receptor (MCH-2R) has been identified. This has much homology to SLC-1 and is expressed in the ARC and VMH (48). The gene encoding MCH-2R has been mapped to a region of chromosome 6 in humans that is associated with obesity (49).
The place of MCH in the hierarchy of hypothalamic pathways that control feeding and body composition is uncertain. It is the only orexigenic hypothalamic peptide known whose loss of expression causes hypophagia and loss of fat (c.f. NPY [15]), but repeated central administration of MCH to rats does not lead to sustained hyperphagia or obesity (50). MCH-overexpressing mice fed a high-fat diet accumulate more body fat than do wild-type littermates eating the same diet (51). This obesity is associated with impaired glucose tolerance and insulin resistance. Thus, MCH may play a more subtle role in determining eating behavior, perhaps involving palatability or the 'reward' aspects of feeding.

Glucose-Sensing Neurons

Glucose is the main metabolic fuel of the brain, and decreases in blood glucose concentration potently stimulate feeding. Specific regions of the central nervous system contain 'glucose-sensing' neurons that can detect changes in glucose availability, notably the PVN, VMH, ARC and LHA, and the NTS. The overall importance of these neurons in energy homeostasis is highlighted by the observation that obesity develops in mice injected with gold-thioglucose, which destroys glucose-sensing neurons in the VMH (52).

Glucose-sensing neurons fall into two classes: glucose-responsive neurons (GRN), which increase their firing rate as glucose levels increase; and glucose-sensitive neurons (GSN), which are stimulated by decreases in glucose (53). Intriguingly, the glucose-sensing machinery of the GRN appears to be very similar to that of the pancreatic \( \beta \) cell (54). It has been postulated that changes in glucose availability are important in the short-term regulation of feeding. A transient, small decrease in blood glucose levels (~0.5 mmol/l) precedes most spontaneous feeding episodes in rats, and giving exogenous glucose at this time can abolish feeding (55). It has therefore been assumed that GRN and GSN are involved in initiating and terminating feeding in response to changes in glucose levels, but this hypothesis has not been rigorously tested.

It is now becoming clear that glucose-sensing neurons are affected by circulating factors other than glucose—for example, leptin and insulin both inhibit the GRN of the VMH (56). Moreover, they also interact with other appetite-regulating neuronal systems: the Orexin neurons establish close and apparently synaptic contact with the glucose-sensing neurons in the LHA (Fig. 4), and Orexin-A potently excites the GSN, suggesting that the two may cooperate in sensing decreases in glucose concentration and perhaps the short-term regulation of feeding (57). Preliminary studies indicate that NPY may also influence glucose-sensing neurons in the LHA.

HOW DOES THE BRAIN SENSE ENERGY REQUIREMENTS?

This is one of the most crucial questions in the understanding of energy homeostasis. Numerous neural pathways have been identified, including those transmitting information about taste, gut distension, and peripheral glucose levels. The vagus
FIG. 4. Close relationship of the processes of a glucose-responsive neuron (center) with Orexin neurons (surrounding elongated profiles).

carries inputs from stretch receptors in the stomach and intestine, and from glucocceptors in the liver that respond to decreases in portal venous glucose concentration, which terminate in the NTS—from where neurons project to the LHA (58).

Various circulating factors have been proposed to signal energy status to the CNS. The role of glucose has been discussed. In addition, leptin and insulin are postulated to be 'adiposity signals' for the long-term regulation of body weight by the brain. Furthermore, the gut's view of nutritional status is transmitted to the brain via signals that include CCK, a novel hormone termed 'ghrelin,' and other brain-gut peptides such as the glucagon-like peptides GLP-1 and GLP-2. Some of the neuronal circuits on which these impact have been identified, but the ways in which these different signals are integrated to provide a complete picture of nutritional state, remain elusive.

Leptin

Leptin (from the Greek leptos, meaning 'thin') is the product of the *ob* gene, identified in late 1994 on mouse chromosome 6 (59). The *ob* gene is highly conserved
among vertebrates. Mouse leptin shares 84% sequence identity to the product of the human \textit{ob} gene, which lies on chromosome 7q31.3 (60). The \textit{ob} gene encodes a single-chain 18-kDa peptide, from which an N-terminal leader sequence is cleaved to liberate a 16-kDa peptide.

Leptin is mainly produced in adipose tissue and secreted into the bloodstream; lower levels of expression are also found in brown adipose tissue (BAT) in rodents, stomach, and placenta (61). The expression and secretion of leptin shows circadian variation and is stimulated by insulin and glucocorticoids, which may explain the fall in expression during fasting (62,63). Two mutations have been identified in the mouse \textit{ob} gene, which both lead to the \textit{ob/ob} syndrome of hyperphagia, obesity and type 2 diabetes: one mutation prevents transcription entirely, while the other (in the coding region) produces a prematurely terminated peptide (61).

The leptin receptors (OB-R), first isolated from mouse choroid, exist as several splice variants that are expressed in various tissues (64). Their generic structure consists of a single protein that spans the cell membrane and resembles the cytokine receptors, notably the gp130 signal-transducing component of the receptor for interleukin-6. Five of the six known leptin receptor isoforms (OB-Ra, OB-Rb, OB-Rc, OB-Rd, and OB-Rf) have transmembrane domains; OB-Re lacks both the transmembrane and intracellular domains and may function as a circulating leptin-binding protein. Only the long isoform (OB-Rb) contains all the intracellular motifs required for efficient activation of the JAK-STAT signal transduction pathway, and is assumed to be the isoform that mainly mediates the effect of leptin on body weight (65).

OB-Rb is highly abundant in the hypothalamus, where it is expressed by the NPY/AGRP and POMC/CART neurons of the ARC, and by MCH and Orexin neurons in the LHA; these neurons, and others in sites including the NTS, appear to mediate some of leptin's central actions, including inhibition of feeding (23). The short OB-Ra isoform of the receptor is found in choroid plexus and kidney (64). The leptin receptor gene maps to chromosome 4 of the mouse and chromosome 5 of the rat, in regions long known to contain mutations that cause two of the classical genetic obesity syndromes in rodents, i.e., the db/db mouse and fa/fa Zucker rat. The db mutation consists of a point mutation (a G→T substitution) that yields a truncated and biologically inactive product, while fa is a single amino-acid change (Gln→Pro) at residue 269 in the extracellular domain. Both mutations interfere with the ability of the brain to 'read' the leptin signal (66,67).

Leptin circulates at levels proportional to body fat mass in rodents, humans, and other mammals (61). When injected either systemically or intracerebroventricularly (at lower dosages) into rodents, leptin decreases food intake and increases BAT activity and whole-body energy expenditure, ultimately causing loss of weight and body fat (61). Circulating leptin can rapidly cross the BBB to enter the mediobasal hypothalamus and may also reach periventricular regions via the cerebrospinal fluid, into which it may be transported by the OB-Ra receptor in the choroid (68). Leptin inhibits the ARC NPY neurons (69), but NPY-knockout transgenic mice also decrease feeding following leptin administration, indicating that leptin also acts on
other neuronal systems (15). Recent functional studies show that leptin inhibits expression of AGRP, MCH, and Orexins, while stimulating the POMC/CART neurons, suggesting that these systems are all important mediators of leptin’s action in the hypothalamus (10).

Absence of biologically active leptin (as in the ob/ob mouse) or failure of the brain to perceive it (the db/db mouse and fa/fa rat) leads to hyperphagia, reduced BAT thermogenic activity and increased fat accumulation. This phenotype is predicted from the known effects of leptin and can be explained by loss of leptin’s normal actions on the major hypothalamic neuronal systems; indeed, the NPY neurons and MCH are overactive in these mutants, while POMC is suppressed.

**Insulin**

Insulin is another well-qualified adiposity signal because it circulates at levels that parallel fat mass and its concentrations increase after feeding. Like leptin, insulin can enter specific brain regions, either by crossing the BBB by a specific transport mechanism (involving insulin receptors on the brain microvessels), or by being transported into the cerebrospinal fluid (70).

Insulin receptors are widely expressed in the brain including the ARC, PVN, olfactory bulb, choroid plexus, hippocampus, cerebral cortex, and brainstem (71). Neurons carry a specific 'brain' variant of the insulin receptor which has the same primary sequence as that found in peripheral tissues, but has a lower molecular weight because it is less heavily glycated; glia express the peripheral-type insulin receptor.

There is much evidence that, like leptin, insulin acts on the brain as a satiety factor to regulate energy balance. ICV or systemic administration of insulin at sub-hypoglycemic dosages in rats reduces food intake and may stimulate thermogenesis, whereas injecting insulin antibodies into the VMH increases feeding (72). The CNS pathways that mediate insulin’s effects on energy balance apparently include the NPY neurons of the ARC because ICV insulin administration inhibits the expression of NPY mRNA (72). In addition to decreased leptin levels, decreases in peripheral insulin may contribute to NPY neuronal overactivity (and thus increased hunger) in fasting and insulin-deficient diabetes, while loss of insulin’s normal inhibitory effect on the NPY neurons—perhaps analogous to insulin resistance in peripheral tissues—may also play a role in the hyperphagia of the fa/fa Zucker rat (73). The relationship between central 'leptin resistance' and 'insulin resistance' is uncertain, but recent evidence suggests that the intracellular signaling pathways for insulin and leptin may converge (74).

**Other Peripheral Signals**

CCK, the archetypal satiety peptide, is released from the gut after eating and an increase in its circulating concentrations terminates feeding (75). CCK appears to operate through both peripheral and central pathways by interacting with different
subclasses of CCK receptor. The satiety effects of CCK are initiated in the periphery via CCKA receptors on vagal afferent fibers in the gut, which relay information to the NTS; vagotomy blocks the hypophagia induced by peripheral CCK administration, underlining the importance of this afferent pathway (76). Central administration of CCK also inhibits feeding, probably by activating central (CCKB) receptors, which are expressed in the VMH and PVN (77). There is evidence that the satiating effect of peripheral CCK acts synergistically with that of other hormones released postprandially, such as insulin, glucagon, and particularly leptin. For example, the anorectic effects of central leptin administration on feeding are blocked by co-injection of CCKA antagonists (78). Intravenous infusion of CCK to achieve physiologic postprandial concentrations decreases hunger and food intake in humans (79).

Ghrelin appears to act in a reciprocal fashion to CCK. It is a 28-residue peptide that was first recognized (and named) for its ability to stimulate growth hormone secretion via the growth hormone secretagogue receptor (80). Ghrelin is principally produced by the stomach and upper small intestine; much lower amounts are expressed in other sites, including the hypothalamus (81), although it remains to be determined whether these serve physiologically relevant functions. Circulating ghrelin levels increase progressively during fasting and decrease after eating, suggesting a role in signaling nutritional state (82). Exogenous administration of ghrelin in rodents stimulates food intake and a reduction in fat utilization, and leads to obesity (83). Growth hormone secretagogue HS receptors have been identified in the major hypothalamic nuclei, notably the mediobasal ARC where ghrelin administration causes activation of the NPY/AGRP neurons (84). Additionally, Y1 receptor antagonists interfere with ghrelin’s orexigenic effects, suggesting that NPY/AGRP neurons play a key role in mediating ghrelin’s effects on energy balance (85). The POMC neurons and MC4-R may also be involved.

A LOOK TO THE FUTURE

As mentioned at the outset, this field is remarkable for both the speed with which knowledge is expanding and the failure of new therapies to emerge from this wealth of data. The most obvious therapeutic indication is obesity, which continues to resist both public health efforts and pharmacologic strategies. Is there any hope that novel treatments for obesity will be developed?

There is evidence that some of the systems previously described operate in man to at least some degree: for example, morbid obesity has been associated in rare cases with mutations in leptin (86), the leptin receptor (87), and various components of the melanocortin axis. These examples provide proof of concept that these factors play some role in human energy homeostasis, but their importance in the common type of lifestyle-related weight gain that accounts for the vast majority of human obesity is far from clear. Recombinant human leptin has proved disappointing in trials in 'common' human obesity, although it has convincingly decreased hyperphagia and body fat mass in one of the very rare cases of genetic leptin deficiency (88).
Numerous possible players are waiting in the wings to see if they can claim their moment of glory in the treatment of a disease that ultimately threatens to claim more lives than mankind’s traditional foes of starvation and infection. Some candidates that appeared promising a few years ago—leptin, NPY Y5 antagonists—now appear to have fallen short of expectation. The next decade will undoubtedly be an exciting time scientifically for those interested in energy homeostasis; we can only hope that the rapidly growing numbers of people who are falling victim to obesity and its sequelae will be able to enjoy tangible benefits of our expanding knowledge.

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**DISCUSSION**

*Dr. Christoph Beglinger:* Can you tell us which two hormones were involved in your sea slug in the picture you showed at the beginning?

*Dr. Gareth Williams:* I think the hormones responsible are given very banal names. One is called the egg-laying hormone and the other one is called the hyperphagic or the eating hormone.

*Dr. Christoph Beglinger:* So then none of these hormones are involved in the current thinking on the control of appetite?

*Dr. Gareth Williams:* I don’t think that these particular two hormones have much relevance to man, a possible exception being medical students in Liverpool. This is a very interesting question though, because a lot of these peptides in the human and rat brain can be traced all the way back down to the lower orders; for example, bombesin, which is a peptide involved undoubtedly in the feeding behavior of the rat was originally described from frog skin.

*Dr. Narendra Kumar Arora:* It’s clear from your presentation that obesity is attributed to both genetic and environmental causes. Can you tell us what proportion is in the environmental surroundings in which we are living, and which may be amenable to intervention.

*Dr. Gareth Williams:* The question is how much obesity you can blame on genes and how much on environment. George Bray, sitting modestly in the second row there has done a vast amount of work on this, and may well have a different answer, but I think the estimates vary. I think it’s fair to say, that genes are currently thought to account for about 40% probably of the total risk of becoming obese. So when the patient says, is it my genes, you’ve got to say yes, sort of. But then you’ve got to tackle the environmental factors, because clearly you can’t do anything about the genes, and using them as an excuse for not changing behavior is not going to get anybody anywhere.

*Dr. Marcello Giovannini:* From your experience, does the amino acid tryptophan influence appetite, for example?

*Dr. Gareth Williams:* We haven’t done any work on the role of amino acids in modulating appetite. Certainly there are effects, and they are often complicated and difficult to interpret and the other thing is to say that these effects are modified, if you give them together with other nutrients—in the same way that the experimental effects of glucose or insulin or leptin on the brain might appear quite pure. When you put them into the context of all the other things that are reaching the brain, particularly after feeding, then I think you have to be a bit circumspect when drawing conclusions.

*Dr. Luciano Tato:* Is there some role for the leptin receptor in the gut?

*Dr. Gareth Williams:* The question is whether there are leptin receptors and a leptin circuit that might help to regulate feeding behavior at the level of the stomach. Now there are leptin receptors there and there has been work to suggest that local leptin production may enhance the satiating effect of cholecystokinin. Whether that’s relevant to man, I don’t think anybody can say at the moment.

*Dr. M. Westerterp-Plantenga:* I have one comment and a question. First of all, I agree with you that administration of recombinant leptin in humans hardly has shown any effects. However, we showed that with a weekly high dose of leptin in addition to a low energy diet, humans lost 25% more weight than the placebo treated group! This was a double-blind randomized placebo-controlled clinical trial. My question is whether these short-term influences may have a longer term effect?

*Dr. Gareth Williams:* In clinical trials, leptin induced weight loss really only at the highest doses and in those trials, injection site reactions with the active compound are really very
common. We know from a lot of other trials that the blinding of treatment (active vs. placebo) is compromised, for example, with an obvious side effect that alerts the patient to the fact that they may be on active treatment. Then those patients are more likely to lose weight, because they believe that they're being actively treated. This is a fundamental problem with real-life, clinical trials. In rodents that eat palatable food and develop dietary obesity, a degree of leptin insensitivity probably occurs at the level of the hypothalamus, perhaps involving NPY and/or POMC neurons. This may help to explain why leptin has so little effect—but until we have a way of measuring a biological response to leptin in man, we aren't going to be able to test hypotheses about whether leptin resistance is a genuine phenomenon in man.

Dr. Wolfgang Langhans: I, too, agree with you that leptin administration in humans generally has only small effects. Some data seem to suggest, however, that leptin may be useful to prevent or attenuate the body weight regain in people who have already lost weight by dieting.

Dr. Ashish Bavdekar: I have a couple of questions. Is there any relationship between leptin and stature, independent of obesity. And secondly, is the relationship between leptin and obesity stronger for general obesity or central obesity?

Dr. Gareth Williams: I'm not aware of any relationship between leptin and height, independently of fat mass. The other question was whether leptin is related more to total body adiposity or just to visceral fat mass. Again, if you look at the literature, there seem to be relationships with both, and the strength of those relationships seems to vary quite a lot between the population and between studies.

Dr. George A Bray: One of the tools that's been used to study this model of food intake is the transgenic animal, where peptides have either been knocked out or over-expressed and you showed leptin, when leptin's deficient, which is really the knockout. You get massively obese and it's the same thing for the melanocortin one, but in all of the cases that I've seen, where the central controllers are involved, they don't alter the susceptibility to a high fat-induced obesity. If you add high fat, you can always make the animal fatter, regardless of whether peptide manipulation is either knockout or overexpression. It suggests that there's a separate control process for dietary fat that seems to be outside the peptides' circuitry you described, and I wondered if you could give me your views about what that is, because that may be a more important component in our design of therapies for people who escape our prevention, than tackling these peptides, which don't seem to provide that dietary susceptibility, at least in the animals I'm aware of.

Dr. Gareth Williams: I've got a couple of observations. The first one is that I'm simply not at all a great believer in the knockout approach in this field. I'm a believer in conditional knockouts, which is where you can turn the gene off at some defined stage and under your control in adult life. But I think that knocking out permanently one strand of a very complicated network of processes that have been deliberately designed to be able to compensate if something goes wrong with one bit may be unhelpful. The molecular geneticists are clearly very clever, but they can also be startlingly naïve, when they come to trying to apply their techniques to this sort of complicated physiology. There have been rather few exceptions to the rule that knockouts don't behave exactly as you'd predict. What is really needed to try and tease these things out is a highly specific, highly selective pharmacological antagonist, which can be given in a reasonable way. And that is complicated, because you would need to have something with a good bioavailability to the brain that would hit only the systems that you wanted to target—in other words, the classical conundrum of drug development. Coming back to the question of whether fat intake is regulated by something else, I don't think we can say at the moment. We've done some work recently with the cannabinoid-1 CCB-1 receptor. Every
other system we've looked at in dietary obesity appears to be altered to compensate for or adapt to the increased fat mass. For example, the MC4 receptors seem to be turned on, especially in animals that don't gain so much weight. Perhaps when you put animals on a high fat diet, they all turn on the POMC, MC4 system a bit. The ones that really succeed in doing that are able to resist overeating the diet and don't get so fat. We also find that the NPY system, which should be stimulating feeding, is turned off. By contrast, the CB1 receptor is the only one that we've found that actually looks as though it might be contributing to driving hypophagia and the appetite for fat, because these receptors behave as though the cannabinoid system which normally acts to stimulate feeding is turned on. As endogenous cannabinoids are involved in determining the palatability of food, this may explain why some animals (and people)—but not others—overeat and get fat when given a palatable, fat-rich diet.

Dr. Veena Kalra: Is there any influence of age on normal leptin levels that can lead one to suppose that obesity coming in early life and then closer to adolescence have different contributions from leptin or is there fairly uniform pattern. Secondly, is the influence of homogenesis associated with alteration in leptin levels different in different disease states, for example in obesity or anorexia-nervosa, or would this be the same for both cases?

Dr. Gareth Williams: The first question concerned the relationship of leptin with age and whether this has any bearing on the different propensity of the different ages of man to develop obesity. There are age-related changes in leptin, but I'm not aware of any systematic data that show that the correlation, or the slope of the line between leptin and the risk of gaining weight varies between the different ages. The second question was about thermogenesis and conditions where weight is reduced. Wolfgang and Anne will be talking about cachexia in various states. Cachexia can be associated with tumors, infections and inflammatory bowel disease and can be induced by administration of various cytokines. In these conditions, the leptin level still parallels with the fat mass, i.e., there are no inappropriate rises in leptin to explain why the animal is not eating and/or is burning off energy excessively. Cachexia is therefore not due to over-production of leptin and the thermogenic effect of leptin doesn't seem to be altered either.

Dr. Vay Liang W. Go: I just want to make a comment. Professor Williams was talking about what proportion of the components of obesity are genetic in origin. I don't want to leave the impression that obesity of genetic cause is not prevention with individuals with genetic polymorphisms. We can prevent cancer of the colon, even in individuals with genetic polymorphisms, but while eating a certain diet. Regarding the second question about the knockout mouse, which we discussed heavily a while ago, the knockout mouse is not the model to be used for obesity research. This is said primarily because there are so many redundancies in the control of feeding and control of satiety.

Dr. Gareth Williams: Your comment is about gene-nutrient interactions, and the fact that you can prevent a lot of cases of colonic cancer even in people with a genetic predisposition by giving them dietary advice. I think it's going to be more complicated with obesity, because this is, par excellence, a multi-genetic, multi-factorial disease. In common obesity there could easily be 12-15 different genes, all interacting variably with each other, with the environment and all having different roles in different populations. Obviously, the environmental components vary widely between populations and within the same population at different ages. In the UK caucasians for example, obesity doubled in prevalence between 1985 and 2000: things are changing so rapidly that they can't be explained by genetic influences alone, and the nature of the interaction between genes in the environment is also changing with time. The data from prevention of colonic cancer are very encouraging, but I'd hesitate to extrapolate
that optimism to the management of obesity. We're going to need a worldwide public health
attack on physical inactivity, including getting rid of cars, televisions, computer games. I'd
argue that those measures are actually going to be more useful than genotyping of somebody
to find that they are at risk from the seventh gene on the list of obesity genes and then giving
them specific nutritional advice.

Dr. Anand Pandit: One of the major worries of developing countries is syndrome X.
Here the BMI is low but the total body fat is pretty high. Now, there has been a lot of talk
between some of us, who have been trying to look at this as a street phenomenon. There are
inflammatory markers like interleukins, and we are thinking of looking into them. Do you
think there is any relationship between inflammatory markers, obesity, and syndrome X?

Dr. Gareth Williams: The question is whether there is any relationship between inflamma-
tory markers and obesity, and I guess with the other aspects of metabolic syndrome, syndrome
X. The problem with the inflammatory markers that have been looked at is that they're all
rather non-specific, such as for example, the cytokines in man. Cytokines are not only produced
during inflammation, but are also expressed in white fat: you're aware of the continuing
debate about whether circulating tumor necrosis factor-α levels mean very much in man or
whether paracrine signaling within fat is more important. I think it's difficult to sort out these
relationships. There is some good agreement obviously between various inflammatory markers
and the risk of atheroma, which may reflect continuing, low-grade inflammation in the arterial
intima. There's certainly some evidence that macrophages in the developing atherosclerotic
plaque produce some of these cytokines, for example.