Hormonal Regulation of Food Intake

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Stanley, Sarah, Katie Wynne, Barbara McGowan, and Stephen Bloom. Hormonal Regulation of Food Intake. Physiol Rev 85: 1131–1158, 2005; doi:10.1152/physrev.00015.2004.—Our knowledge of the physiological systems controlling energy homeostasis has increased dramatically over the last decade. The roles of peripheral signals from adipose tissue, pancreas, and the gastrointestinal tract reflecting short- and long-term nutritional status are now being described. Such signals influence central circuits in the hypothalamus, brain stem, and limbic system to modulate neuropeptide release and hence food intake and energy expenditure. This review discusses the peripheral hormones and central neuronal pathways that contribute to control of appetite.

I. INTRODUCTION

The brain regulates energy homeostasis in response to signals from both adipose tissue and the gastrointestinal tract. The drive to eat and energy expenditure are adjusted so that over time, body weight remains stable.

Over the past decade, our knowledge of this homeostatic system has increased dramatically. Important advances have been made in the characterization of hypothalamic neuronal networks and neuropeptide transmitters, along with the discovery of circulating peptides that send signals to the brain regarding the body’s nutritional status (see Fig. 1).

Disorders of this essential homeostatic mechanism lead to obesity and its associated complications. Currently, the prevalence of obesity is increasing unabated, bringing with it significant morbidity and mortality. The understanding of the physiological systems regulating food intake and body weight is fundamental to establishing effective therapies for this world-wide epidemic.

II. PERIPHERAL REGULATORS OF APPETITE

A. Adipose Tissue Hormones

1. Leptin

Although originally thought to be inert tissue solely for the storage of energy, it has now become clear that adipose tissue is an active endocrine organ. One of its most important hormones is leptin, a peptide hormone with numerous actions, including influences on energy homeostasis and neuroendocrine and immune function. Leptin is the product of the ob gene expressed predominantly in adipocytes (458) but also at lower levels in gastric epithelium (23) and placenta (267). Circulating leptin levels reflect both energy stores and acute energy balance. Plasma leptin levels are highly correlated with adipose tissue mass (258), but food restriction results in suppression of circulating leptin (143, 258), which can be reversed by refeeding or insulin administration. Exogenous leptin administration, both centrally and peripherally, reduces spontaneous and fasting-induced hyperpha-
gia (7) whilst chronic peripheral administration reduces food intake resulting in loss of fat mass and body weight (168).

Leptin signals via a single-transmembrane domain receptor of the cytokine receptor family (394). Alternative mRNA splicing and posttranslational processing result in multiple isoforms of the receptor (Ob-R) (75, 393). The alternate splice variants of the receptor may be classified into three forms: long, short, and secreted (150, 393). The long form, Ob-Rb, receptor possesses a long intracellular domain that binds to JAK-kinases (238) and to STAT 3 transcription factors (411) resulting in signal transduction and leptin’s effects on food intake (238). Activation of the JAK-STAT pathway induces expression of suppressor of cytokine signaling-3 (SOCS-3), one of a family of cytokine-inducible inhibitors of signaling. SOCS-3 expression is upregulated by leptin in hypothalamic nuclei expressing the Ob-Rb receptor. Overexpression of SOCS-3 blocks leptin’s actions on a reporter gene construct in vitro and, therefore, obesity-related leptin resistance has been postulated to be a consequence of increased or excessive SOCS-3 expression. Consistent with this hypothesis, neuron-specific conditional deletion of SOCS-3 in mice results in resistance to diet-induced obesity (291). Similarly, mice heterozygous for global SOCS-3 deficiency are resistant to weight gain and more sensitive to the weight-reducing effect of exogenous leptin administration (193). Thus suppression of SOCS-3 expression may be a potential treatment of leptin-resistant obesity.

Circulating leptin crosses the blood brain barrier (BBB) via a saturable process (28), and it has been proposed the short forms of the receptor play a role in this transport of leptin (121). The secreted (or soluble) form of the leptin receptor is thought to bind circulating leptin, thus modulating its biological availability and hence activity (150).

The long form of the leptin receptor, Ob-Rb, is expressed widely within the hypothalamus but is found particularly in the arcuate nucleus (ARC), ventromedial and dorsomedial hypothalamus (VMH and DMH, respectively), lateral hypothalamic area (LHA) and medial preoptic area (MPOA) (128, 135, 167). Ob-Rb are also expressed in appetite-modulating pathways in the brain stem (277). Peripheral leptin administration alters neuronal activity in these hypothalamic and brain stem regions (127). In the ARC, Ob-Rb mRNA is expressed by the two major neuronal groups: neurons expressing the orexigenic neuropeptides neuropeptide Y (NPY) and agouti related peptide (AgRP) (276) and also by neurons expressing proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) (73). Leptin inhibits the activity of orexigenic NPY/AgRP neurons and reduces expression of NPY and AgRP (122, 166, 357, 379) whilst leptin activates anorectic POMC/CART neurons. Thus, in conditions of low circulating leptin, such as food restriction, NPY and AgRP expression are upregulated and the orexigenic NPY/AgRP neurons are activated, while in times of plenty, with high plasma leptin, the anorectic pathways mediated by POMC and CART are switched on. Although Ob-Rb are expressed in many hypothalamic nuclei, the actions of leptin may differ between these hypothalamic regions. With the use of viral-mediated gene expression, chronic leptin overexpression in the ARC, PVN, and VMH resulted in reduced food intake and energy expenditure. However, leptin overexpression in the MPOA did not alter food intake but did reduce energy expenditure (25).

The absence of leptin has profound effects on body weight. Lack of circulating leptin, due to a mutation in the ob gene, leads to hyperphagia, obesity, as well as neuroendocrine and immune disturbance in the ob/ob mouse, which can be normalized by leptin administration (64,
Similarly, human leptin deficiency in both children and adults causes severe obesity and hypogonadism (284, 382), which can be ameliorated by recombinant leptin therapy (132, 246). In addition to its effects on food intake, leptin also modulates energy expenditure in rodents (though not in humans); the hypothalamo-pituitary control of the gonadal, adrenal, and thyroid axes (7, 67); and the immune response (251). Thus the body’s response to a decrease in energy stores appears to be integrated by leptin to the etiology of obesity. Lack of sensitivity to the anorectic actions of central leptin administration can predict the later development of obesity in rodents on a high-energy diet (244). Furthermore, a high-fat diet itself, before changes in body composition, may induce leptin resistance, since rodents placed on a high-fat diet have an attenuated response to leptin administration even before weight gain (250).

Thus, although leptin deficiency has profound effects on food intake, body weight, and endocrine function, the high leptin levels found in obese individuals are much less effective at reversing weight gain. Thus leptin’s primary role may be as a hormone of starvation rather than one of plenty.

2. Adiponectin

Adiponectin, also called adipocyte complement-related protein (Acrp30), apM1 or adipoQ, is a 244-amino acid protein secreted from adipose tissue. Its circulating levels are up to 1,000-fold higher than other circulating hormones such as leptin and insulin (401). Adiponectin has four domains: a cleaved amino-terminal signal sequence, a region with no homology to other known proteins, a collagen-like region, and a carboxy-terminal globular domain. The globular domain forms homotrimers, and additional interactions with collagenous segments cause the formation of higher molecular weight complexes (315). The globular domain shares a sequence homology with several proteins, including the complement factor protein C1q and tumor necrosis factor-α (TNF-α).

The function of adiponectin is largely unknown but is postulated to regulate energy homeostasis (353). The plasma concentration of adiponectin is inversely correlated with adiposity in rodents, primates, and humans (16, 192, 194). Adiponectin is significantly increased after food restriction in rodents (39) and after weight loss induced by a calorie-restricted diet (191) or gastric partition surgery in obese humans (449). Peripheral administration of adiponectin to rodents has been shown to attenuate body weight gain, by increased oxygen consumption, without affecting food intake (39, 145, 448). The effect of peripheral adiponectin on energy expenditure seems to be mediated by the hypothalamus, since adiponectin induces early gene c-fos expression in the PVN and may involve the melanocortin system (325). It is perhaps counterintuitive for a factor that increases energy expenditure to increase following weight loss; however, reduced adiponectin could perhaps contribute to the pathogenesis of obesity.

Studies show that plasma adiponectin levels negatively correlate with insulin resistance (192), and treatment with adiponectin can reduce body weight gain, increase insulin sensitivity, and decrease lipid levels in rodents (39, 325, 448). Adiponectin knock-out mice demonstrate severe diet-induced insulin resistance (256) and a propensity toward atherogenesis in response to intimal injury (224). Thus adiponectin, as well as increasing energy expenditure, may also provide protection against insulin resistance and atherogenesis.

The mechanism by which adiponectin improves insulin resistance, glucose metabolism, and attenuation of weight gain is not yet fully understood, although some of these effects may be mediated through metabolic pathways that include regulation of food intake, gluconeogenesis, and lipogenesis (368). Of interest, peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists, the thiazolidinediones, can increase circulating adiponectin levels in both rodent models of obesity (83) and in...
patients with obesity/type II diabetes mellitus (257). Indeed, chronic transgenic expression of adiponectin causes effects that are similar to those of chronic treatment with thiazolidinediones, suggesting that part of the insulin-sensitizing effects of thiazolidinediones may be mediated by an increase in adiponectin levels (82).

Recently, two distinct adiponectin receptors have been cloned (447). The first, adipOR1, is highly expressed in skeletal muscle, has a high affinity for the globular domain of Acrp30 (gAcrp30), and a has low affinity for the full-length ligand. The second, adipOR2, is highly expressed in the liver and shows preferential binding to the full-length ligand. This is consistent with earlier reports that show a differential effect of gAcrp30 and the full-length ligand in muscle and liver. Adiponectin receptors have also been detected in the brain, and more specifically in the hypothalamus (325).

3. Resistin

Resistin is produced by adipose tissue and appears to increase insulin resistance. Circulating resistin is increased in obese rodents (380) and falls after weight loss in humans (412). Recent studies suggest that resistin knockout mice show increased glucose tolerance with a high-fat diet (385). Transgenic mice overexpressing a dominant negative form of resistin showed increased adiposity with elevated leptin and adiponectin levels, as well as enhanced glucose tolerance and insulin sensitivity (385). Although resistin may contribute to the development of insulin resistance and diabetes in obesity (380), its role in the pathogenesis of obesity remains to be defined.

B. Pancreatic Hormones

1. Insulin

The pancreatic hormone insulin was one of the first adiposity signals to be described (358) and, like leptin, is positively correlated with long-term energy balance (24, 437). Plasma insulin concentrations depend on peripheral insulin sensitivity, which is related to both total body fat stores and fat distribution, with visceral fat being a key determinant (324). However, unlike leptin levels, which are relatively insensitive to acutely increased food intake, insulin secretion increases rapidly after a meal (323).

There is considerable evidence that insulin acts as an anorectic signal within the central nervous system (CNS). Centrally administered insulin or an insulin mimetic decreases food intake and body weight (8) and alters expression of hypothalamic genes known to regulate food intake. Insulin infusion into the third cerebral ventricle in rodents (198) or lateral ventricle in primates (438) dose-dependently decreases food intake resulting in weight loss over a period of weeks. Intrahypothalamic (PVN) insulin injection also decreases food intake and weight gain in rats (275). Treatment with novel, orally available insulin mimetics also decreases weight gain, adiposity, and insulin resistance in mice on a high-fat diet (8). Conversely, antibodies to insulin injected into the VMH of rats stimulate food intake (383), and repeated administration of antisera increases food intake and rate of weight gain (270). Administration of antisense RNA against the insulin receptor precursor protein results in hyperphagia and increased fat mass (309). Similarly, neuron-specific deletion of the insulin receptor results in obesity, hyperinsulinemia, and dyslipidemia in male mice (56).

Insulin enters the CNS via saturable, receptor-mediated uptake across the BBB at levels proportional to circulating insulin concentrations (34). Little or no insulin is synthesized within the brain (27, 439). Therefore, peripheral insulin should have actions similar to central insulin administration. Studies of systemic insulin administration are complicated by hypoglycemia, which in itself potently stimulates food intake, but hyperinsulinemic, euglycemic clamp studies have indeed shown a reduction in food intake in both rodents and baboons (305, 440).

Insulin signals via a cell-surface insulin receptor, which is composed of an extracellular, ligand binding α-subunit and an intracellular β-subunit with intrinsic tyrosine kinase activity. There are two splice variants of the insulin receptor: subtype A with greater affinity for insulin and widespread expression and subtype B with lower affinity and expression in classical insulin-responsive tissues such as fat, muscle, and liver. Insulin receptors are widely distributed in the brain, particularly in hypothalamic nuclei involved in food intake (ARC, DMH, PVN, suprachiasmatic and periventricular regions) (88, 263). Insulin receptor activation is via several insulin receptor substrates (IRSs), which include IRS-1 and IRS-2 (30, 58). Although IRS-1 null mice show no differences in food intake or body weight from their wild-type littermates (14), IRS-2 null mice have increased food intake, increased fat stores and infertility (58). IRS-2 mRNA is highly expressed in the ARC, and therefore, insulin’s central actions may be mediated by IRS-2 (58). Insulin and leptin, along with other cytokines, appear to share common intracellular signaling pathways. Both may signal via IRS and the enzyme phosphatidylinositol (PI) β-kinase (306, 324), allowing intracellular integration of their appetite-regulating actions.

The pathways mediating insulin’s effects on food intake remain to be fully elucidated. Hypothalamic NPY may be an effector of insulin’s actions. Intracerebroventricular insulin administration prevented the fasting-induced increase in NPY mRNA expression in the PVN and ARC in rats (360). NPY expression is also increased in insulin-deficient, streptozotocin-treated rats but restored by insulin replacement (428, 435). The melanocortin sys-
Pancreatic polypeptide (PP) is a member of the PP-fold family of peptides which also includes peptide YY (PYY) and NPY. They have significant sequence homology including several tyrosine residues (85). They share a common tertiary structure, an α-helix and polyproline helix, connected by a β-turn to produce a characteristic U-shape, the PP-fold (157).

PP is primarily produced by cells at the periphery of the islets of Langerhans but is also secreted by the exocrine pancreas and distal gastrointestinal tract (233). Plasma PP concentrations show diurnal variation, with lowest levels in the early hours of the morning and highest in the evening (400). In addition to this, circulating PP concentrations rise following food intake and remain elevated for ~6 h (4). Postprandial release is biphasic, and although total release is proportional to caloric intake, the contribution of the first phase increases with consecutive meals (400). Circulating PP levels are also elevated by ghrelin, motilin, and secretin and by gastric distension (18, 74, 281, 319) and reduced by somatostatin administration (316). Plasma PP concentrations have been suggested to be inversely proportional to adiposity, with elevated levels in anorexic subjects (147, 408) and both reduced levels (155, 234) and attenuated second phase release in obese subjects (234). Subjects with obesity due to Prader-Willi syndrome have reduced basal and blunted postprandial PP release, which may contribute to their hyperphagia and obesity (460, 461). However, others report no difference in plasma PP concentrations between lean and obese subjects (436) or following weight loss in lean and obese subjects (42). Postprandial PP (20). Thus PP sends anorectic signals via brain stem pathways, regulation of hypothalamic neuropeptides, and by modulating expression of other gut hormones.

In contrast to the peripheral actions of PP, PP administered into the third ventricle increases food intake (76). However, the receptors mediating this action and the mechanisms involved are unclear.

C. Gut Hormones

1. PYY

PYY is released from the L cells of the gastrointestinal tract, with increasing tissue concentrations found in the more distal portions, the ileum, colon, and rectum (5, 120). PYY release is correlated with calorie intake, with levels rising to a plateau 1–2 h after a meal and remaining elevated for 6 h (5). Interestingly, the increase in plasma PYY concentrations is seen rapidly after food intake, well before nutrients are in contact with the L cells of the distal intestine. This suggests initial PYY release may be the consequence of a neural reflex though direct contact with nutrients may play a role later (146). Macronutrient composition of food, in addition to total calories, influences circulating PYY concentrations: isocaloric intake of fat elicits a greater rise in plasma PYY than consumption of protein or carbohydrate (249). Circulating PYY levels are also influenced by other signals; gastric acid, cholecystokinin and luminal bile salts, insulin-like growth fac-
PYY in the circulation exists in two major forms: PYY<sub>1-36</sub> and PYY<sub>3-36</sub> (158). PYY<sub>3-36</sub> binds with greatest affinity at the presynaptic autoinhibitory Y<sub>2</sub> receptor and is thus a potent, peripherally active anorectic signal. It is the product of cleavage of the amino terminus Tyr-Pro residues by dipeptidyl peptidase IV (DPP-IV) from PYY<sub>1-36</sub> (116). DPP-IV is involved in the cleavage of multiple hormones including products of the proglucagon gene (48). However, the accurate proportions of PYY<sub>1-36</sub> and PYY<sub>3-36</sub> in the circulation in fasting and following food intake remain to be determined.

Peripheral administration of PYY has numerous actions. It delays gastric emptying, delays pancreatic and gastric secretions, and increases ileal absorption of fluids and electrolytes (6, 9, 185). Peripheral PYY<sub>3-36</sub> administration also inhibits food intake and reduces weight gain in mice, rats, and primates (32, 66, 290) and improves glycemic control in rodent models of diabetes (322). PYY<sub>3-36</sub> is also effective in reducing food intake in humans. Intravenous administration of PYY<sub>3-36</sub> reduced food intake by 30% and also reduced subjective hunger in normal-weight human subjects (31, 32). Interestingly, this effect is seen for up to 12 h after the PYY<sub>3-36</sub> infusion has finished and long after circulating PYY<sub>3-36</sub> has returned to basal levels (32). These data suggest PYY<sub>3-36</sub> may be a physiologically important postprandial satiety signal.

Unlike PP, PYY is able to cross the BBB by transmembrane diffusion from the circulation (307). Evidence suggests the anorectic effect of peripheral PYY<sub>3-36</sub> may be mediated via the presynaptic inhibitory Y<sub>2</sub> receptor present on arcuate NPY neurons (54). PYY<sub>3-36</sub> inhibits activity of over 90% of all arcuate neurons and reverses fasting-induced c-fos expression in the arcuate nucleus (334). In particular, PYY inhibits NPY neurons (32) and reduces hypothalamic NPY mRNA expression (32, 66). Moreover, the anorectic effect of PYY<sub>3-36</sub> is absent in Y<sub>2</sub> receptor knockout mice and diminished by a selective Y<sub>2</sub> antagonist (32). Reduction in NPY neuronal activity also increases activation of arcuate neurons expressing POMC, which may contribute to reduced food intake.

Although peripheral PYY<sub>3-36</sub> administration induces expression of the early gene, c-fos, in POMC neurons (32, 169) and increases arcuate POMC mRNA expression (66), the melanocortin system does not appear to be vital for PYY’s effects on appetite. PYY<sub>3-36</sub> is equally effective at reducing food intake in MC4R knockout mice (169) and POMC null mice (65). There is some evidence to suggest a role for CART in mediating the effect of PYY<sub>3-36</sub> on appetite (80). However, peripheral administration of PYY<sub>3-36</sub> also reduces plasma ghrelin levels (31), which may contribute to its anorectic effect. However, the anorectic effect of PYY<sub>3-36</sub> does appear to depend on minimization of environmental stress (169) and therefore some have found its actions difficult to reproduce (402). Both stress and PYY<sub>3-36</sub> act via the arcuate nucleus to alter food intake (86, 260). When appetite is inhibited by stress, no further inhibition can occur with PYY<sub>3-36</sub> administration. Because rodents are easily stressed, inappropriate experimental conditions would mask the anorectic effect of PYY<sub>3-36</sub> leading to the variability reported.

The role of PYY in regulation of body weight is less clear. In rodents, chronic peripheral administration of PYY<sub>3-36</sub> reduced weight gain (32). Obese humans have reduced plasma PYY levels and a relative deficiency of postprandial secretion (243), which might contribute to the maintenance of their obesity. However, obese subjects remain sensitive to the anorectic actions of exogenous PYY<sub>3-36</sub> administration. In addition, vertical banded gastroplasty (11) or jejunoileal bypass surgery (302) raises plasma PYY levels in obese patients, and this may contribute to their appetite loss. Thus long-term administration of PYY<sub>3-36</sub> has the potential to be an effective obesity therapy.

In contrast to peripheral PYY<sub>3-36</sub> centrally administered PYY<sub>1-36</sub> and PYY<sub>3-36</sub> increase food intake. PYY injection into the third, lateral or fourth cerebral ventricles (77, 89), the PVN (376), or the hippocampus (163) potently stimulates food intake in rodents. However, this effect is reduced in both Y<sub>1</sub> and Y<sub>5</sub> receptor knockout mice (205). Therefore, while circulating PYY<sub>3-36</sub> may access the higher affinity ARC Y<sub>2</sub> receptors (32), the central feeding effects of PYY<sub>1-36</sub> and PYY<sub>3-36</sub> may be mediated by the lower affinity Y<sub>1</sub> and Y<sub>5</sub> receptors.

2. Ghrelin

Ghrelin is the endogenous agonist of the growth hormone secretagogue receptor (GHS-R) and a potent orexigenic factor. It is produced and released primarily by the gastric oxyntic cells; however, total gastrectomy only reduces plasma ghrelin by 50–60%. The remaining circulating ghrelin is released by the duodenum, ileum, cecum, and colon (103, 342). Ghrelin is a 28-amino acid peptide with addition of an acyl side chain, n-octanoic acid, to the third serine residue. This octanoylation is essential for binding to the GHS-R type 1a and for ghrelin’s effects on food intake (219).

Plasma ghrelin levels are regulated both by an endogenous diurnal rhythm and by food intake. In rats, ghrelin peaks at the end of the light and dark periods (296). In humans, ghrelin levels are in phase with the diurnal variation in leptin, which is high in the morning and low at night (98). In humans with fixed meal times, plasma ghrelin is greatest during fasting and falls after food intake (17, 98, 404). The postprandial reduction in circulating ghrelin is regulated both by calorie intake and circulating
nutritional signals, such as glucose (342, 403). In rodents, plasma ghrelin and gastric ghrelin mRNA fall after ingestion of either fat or carbohydrate. However, the suppression observed after fat intake is transient and has returned to normal after 45 min, unlike the longer lasting fall after carbohydrate consumption (345). Interestingly, plasma ghrelin levels do not fall after ingestion of water, suggesting gastric distension does not inhibit ghrelin release (403). Energy stores also regulate ghrelin. Circulating ghrelin is inversely correlated with adiposity. Thus anorexics have high plasma ghrelin, which normalizes after weight gain (314). Conversely, obese individuals have reduced plasma ghrelin, which rises to normal after diet-induced weight loss (99, 172). Obese subjects appear to have altered postprandial regulation of ghrelin; they do not show the rapid postprandial fall in circulating ghrelin, and this in turn may play a role in continued food intake and obesity (130). The contribution of ghrelin gene polymorphisms to obesity remains controversial (184, 419). Although there are reports of polymorphisms associated with early-onset obesity (220, 280), other polymorphisms have been found to be protective against fat accumulation (409).

An increase in circulating ghrelin levels may occur as a consequence of the anticipation of food, or may have a physiological role to initiate feeding. Peripheral or central ghrelin administration increases food intake and body weight and reduces fat utilization in rodents (403, 442). Furthermore, blockade of ghrelin’s actions by central infusion of anti-ghrelin antibodies attenuates fasting-induced refeeding, suggesting ghrelin is an endogenous regulator of food intake (298). Ghrelin also increases food intake in humans. Intravenous ghrelin increased food intake by 28% in healthy subjects (441). In addition, rising preprandial plasma ghrelin levels correlate with hunger scores in humans eating spontaneously (97). The severe hyperphagia in subjects with Prader-Willi syndrome is associated with markedly elevated circulating ghrelin, in contrast to most obese individuals who have suppressed plasma ghrelin (96). Bariatric surgery reduces plasma ghrelin despite weight loss, and this may contribute to the appetite suppression and continued weight loss following this treatment (99). However, Callahan et al. (62) did not demonstrate any correlation between ghrelin levels and spontaneous eating in humans. Similarly, altering the feeding schedule in sheep modulates the timing of ghrelin peaks (384). These data suggest the preprandial rise in ghrelin may be a conditioned response possibly to prepare the metabolism for an influx of calories.

Ghrelin’s actions on food intake are thought to be mediated via the growth hormone secretagogue receptor (GHS-R) type 1a. Ghrelin administration does not increase food intake in GHS-R type 1a null mice (70, 387). Ghrelin also increases growth hormone (GH) release via this receptor in the hypothalamus (104, 219, 403, 443). However, the orexigenic action of ghrelin is seen in GH-deficient mice and therefore independent of its GH releasing effects (366, 390, 403). GHS-R type 1a is expressed in numerous tissues: hypothalamus, pituitary, myocardium, stomach, small intestine, pancreas, colon, adipose tissue, liver, kidney, placenta, and peripheral T cells (103, 106, 160, 176, 297). There are studies describing ghrelin analogs that show dissociation between the feeding effects and stimulation of GH, suggesting GHS-R type 1a may not be the only receptor mediating the effects of ghrelin on food intake (398).

Ghrelin’s actions on food intake are probably via the ARC nucleus of the hypothalamus. Peripheral ghrelin administration increases c-fos in ARC NPY neurons (420), and ghrelin fails to increase food intake following ablation of the ARC (390) or in knock-out mice lacking both NPY and AgRP signaling (70). However, the brain stem may also mediate ghrelin’s actions, since GHS-R are expressed on the vagus nerve (105) and ghrelin administration increases c-fos in the nucleus of the solitary tract (NTS) and area postrema (237, 298).

Although the majority of ghrelin is synthesized in the periphery, ghrelin is also expressed centrally. Ghrelin immunoreactive neurons are found adjacent to the third ventricle and lie between the DMN, VMH, PVN, and ARC. These ghrelin neurons have terminals on hypothalamic NPY/AgRP, POMC, and corticotrophin-releasing hormone (CRH) neurons and may activate ARC NPY neurons to form a central circuit regulating energy homeostasis (93). In addition, the hypothalamic ghrelin neurons also terminate in the LHA on neurons expressing orexin (399). Central ghrelin administration stimulates orexin-expressing neurons (237, 399), and central ghrelin-stimulated food intake is attenuated after administration of anti-orexin antibody and in orexin null mice (399). However, the physiological roles of peripheral and central ghrelin remain to be fully elucidated.

Although ghrelin has potent actions on food intake in animals and humans, both ghrelin null mice and mice lacking GHS-R type 1a have normal appetite and body composition on a standard diet (70, 386, 387). This absence of phenotype suggests that long-term ghrelin blockade may not alter body weight, and ghrelin receptor antagonists may not be an effective therapy for obesity.

3. GLP-1

The preproglucagon gene product is widely expressed in the L cells of the small intestine, in the pancreas, and in the brain stem NTS (392). Tissue-specific cleavage of proglucagon by the enzymes prohormone convertase 1 and 2 results in different products (186). Glucagon is the major product in the pancreas, whereas in the CNS and intestine, the major products are GLP-1 and GLP-2 and oxyntomodulin (OXM).
GLP-1 is released by the L cells of the small intestine following nutrient ingestion (180), and circulating GLP-1 levels are inversely correlated with body mass (188, 300, 328, 417). GLP-1 acts to inhibit food intake. Acute GLP-1 injection into the third or fourth ventricles or into the PVN reduces calorie intake (406), and chronic central administration decreases weight gain in rodents (273). Peripheral injection also reduces food intake and activates c-fos in the brain stem (392, 445). Thus peripheral GLP-1 may influence energy homeostasis via the brain stem. In humans, GLP-1 dose-dependently decreases food intake (416). However, when the infusions mimic postprandial concentrations, the effect is small (139, 417). Despite reported reduced GLP-1 levels in obesity, obese subjects remain sensitive to the anorectic actions of GLP-1 (416). Preprandial subcutaneous GLP-1 injection reduced calorie intake by 15% and resulted in 0.5 kg weight loss over 5 days in obese individuals (299). Therefore, low circulating GLP-1 could contribute to the pathogenesis and maintenance of obesity, and GLP-1 replacement could restore satiety.

GLP-1 is a powerful incretin hormone, potentiating all stages of insulin biosynthesis (221, 255). Both short-term intravenous GLP-1 infusion (303) and 6-wk subcutaneous GLP-1 infusion (454) are effective at normalizing blood glucose in poorly controlled type 2 diabetes. Although not a primary end point, subcutaneous infusion also reduced body weight by 2 kg over the 6-wk period (454). However, GLP-1 has been reported to result in hypoglycemia in nondiabetic subjects (397), which may limit its usefulness as an obesity therapy. In addition, GLP-1's use as an obesity treatment may be hampered by its very short half-life, as it is rapidly broken down by the enzyme DPP-IV. However, albumin-bound GLP-1, which is resistant to DPP-IV, the GLP-1 receptor agonist exendin-4 forms derived from the same gene product: CCK-58, CCK-20 and 44% in rodents and humans, respectively (79, 101). GLP-1's use as an obesity treatment may be hampered by its very short half-life, as it is rapidly broken down by the enzyme DPP-IV. However, albumin-bound GLP-1, which is resistant to DPP-IV, the GLP-1 receptor agonist exendin-4 forms derived from the same gene product: CCK-58, CCK-20 and 44% in rodents and humans, respectively (79, 101).

5. Cholecystokinin

Cholecystokinin (CCK) is expressed widely in the gastrointestinal tract (232) but is found particularly in the duodenum and jejunum. There are multiple bioactive forms derived from the same gene product: CCK-58, CCK-33, and CCK-8 (330). CCK is released locally and into the circulation following nutrient ingestion. Its release is rapid, and plasma levels remain elevated for up to 5 h (247). CCK is also expressed within the CNS, acting as a neurotransmitter regulating reward behavior, anxiety, memory, and satiety (95).

The role of CCK in regulation of digestion and appetite has long been known. It stimulates pancreatic and gall bladder enzyme release, inhibits gastric emptying, and increases intestinal motility (247, 289). CCK acts rapidly to reduce meal size and duration in both humans and animals (153, 217), and this effect is potentiated by gastric distension (216). However, CCK has a half-life of only 1-2 min, and its effects are short-lived. It is ineffective if given more than 15 min before food (153). In animals, repeated CCK administration does not alter body weight for although meal size is reduced, meal frequency increases and there is no overall change in intake (425, 426). Similarly, continuous CCK infusion is not effective after the first 24 h (94). However, the OLETF rat, which lacks CCKA.

GLP-1 is released by the L cells of the small intestine following nutrient ingestion (180), and circulating GLP-1 levels are inversely correlated with body mass (188, 300, 328, 417). GLP-1 acts to inhibit food intake. Acute GLP-1 injection into the third or fourth ventricles or into the PVN reduces calorie intake (406), and chronic central administration decreases weight gain in rodents (273). Peripheral injection also reduces food intake and activates c-fos in the brain stem (392, 445). Thus peripheral GLP-1 may influence energy homeostasis via the brain stem. In humans, GLP-1 dose-dependently decreases food intake (416). However, when the infusions mimic postprandial concentrations, the effect is small (139, 417). Despite reported reduced GLP-1 levels in obesity, obese subjects remain sensitive to the anorectic actions of GLP-1 (416). Preprandial subcutaneous GLP-1 injection reduced calorie intake by 15% and resulted in 0.5 kg weight loss over 5 days in obese individuals (299). Therefore, low circulating GLP-1 could contribute to the pathogenesis and maintenance of obesity, and GLP-1 replacement could restore satiety.

GLP-1 is a powerful incretin hormone, potentiating all stages of insulin biosynthesis (221, 255). Both short-term intravenous GLP-1 infusion (303) and 6-wk subcutaneous GLP-1 infusion (454) are effective at normalizing blood glucose in poorly controlled type 2 diabetes. Although not a primary end point, subcutaneous infusion also reduced body weight by 2 kg over the 6-wk period (454). However, GLP-1 has been reported to result in hypoglycemia in nondiabetic subjects (397), which may limit its usefulness as an obesity therapy. In addition, GLP-1’s use as an obesity treatment may be hampered by its very short half-life, as it is rapidly broken down by the enzyme DPP-IV. However, albumin-bound GLP-1, which is resistant to DPP-IV, the GLP-1 receptor agonist exendin-4 forms derived from the same gene product: CCK-58, CCK-20 and 44% in rodents and humans, respectively (79, 101). GLP-1’s use as an obesity treatment may be hampered by its very short half-life, as it is rapidly broken down by the enzyme DPP-IV. However, albumin-bound GLP-1, which is resistant to DPP-IV, the GLP-1 receptor agonist exendin-4 forms derived from the same gene product: CCK-58, CCK-20 and 44% in rodents and humans, respectively (79, 101).

5. Cholecystokinin

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receptors (but not the CCK\textsubscript{A} receptor knockout mouse), is hyperphagic and obese (286, 356). Similarly, chronic administration of CCK\textsubscript{A} antagonists or anti-CCK antibodies increases weight gain in rodents, although without a significant change in food intake (272, 274). The long-term effect of CCK on body weight may be the result of interaction with other signals of adiposity such as leptin, since leptin enhances the satiating effect of CCK (268). The evidence for a role of CCK in long-term body weight regulation, and hence as a potential therapy for obesity, remains contradictory.

CCK acts via seven transmembrane domain G protein-coupled receptors, CCK\textsubscript{A} and CCK\textsubscript{B} (421). CCK\textsubscript{A} receptors are expressed widely in the CNS including the NTS, DMH, and area postrema, while in the periphery they are present in the pancreas and on vagal afferent and enteric neurons. CCK\textsubscript{B} receptors are also found on the afferent vagus nerve and within the stomach and are present widely in the CNS (287, 288, 421, 422). The effects of CCK on appetite are thought to be via the CCK\textsubscript{A} receptor subtype (21). Only the sulfated form of CCK, which binds with high affinity to CCK\textsubscript{A} receptors, inhibits food intake (153). Furthermore, food intake is increased and satiety reduced by administration of a CCK\textsubscript{A} receptor antagonist (36, 182). Peripheral CCK may act directly on the CNS by crossing the BBB (331). Evidence from the CCK\textsubscript{A} receptor knock-out (OLETF) rat suggests that CCK may act on the DMH to suppress NPY levels (45), and administration of CCK into the DMH reduces food intake (47). Activation of the vagus is also important in mediating the actions of CCK on satiety (285, 355). This action may be in part via a paracrine or neurocrine effect with locally released CCK activating vagal fibers without significant alteration of circulating CCK level (332). Activation of the vagus in turn activates the NTS, which then relays information to the hypothalamus (361).

6. Bombesin

Bombesin is a tetradecapeptide originally isolated from amphibian skin (50). Bombesin-like immunoreactivity is widely distributed in the mammalian gut, and plasma levels have been shown to increase sharply after feeding (152). Bombesin is similar in structure to mammalian gastrin-releasing peptide (GRP) and neuromedin B (50). It binds to three different receptors: a GRP receptor, a neuromedin B receptor, and a bombesin-3 receptor (228). Peripheral or central injections of bombesin reduce food intake that is not blocked by vagotomy (152, 374), and its effect is independent of CCK (248). A bombesin-3 receptor knock-out mouse is moderately obese at 6–8 wk of age, but hyperphagia is only significant 12 wk after obesity has developed (310).

III. CENTRAL REGULATORS OF APPETITE

A. Hypothalamic Structure and Neuronal Pathways Regulating Appetite

Despite wide daily variation in food intake and energy expenditure, for most individuals, body weight remains remarkably stable over long periods of time. For this, food intake and energy expenditure must be constantly modulated and balanced. The hypothalamus is essential for the regulation of appetite and energy balance. Hetherington and Ranson (181) and Anand and Brobeck (12) first proposed a model of lateral hypothalamic feeding centers and ventromedial hypothalamic satiety centers. Lesions of the LHA decrease food intake and eventually lead to starvation and death. In contrast, lesions of several of the mediobasal hypothalamic nuclei result in obesity, decreased activity, and neuroendocrine abnormalities. Destruction of the ARC with systemic monosodium glutamate produces obesity and hyperphagia (312), while lesions of the VMN also result in increased body weight and central hypogonadism (108). Similarly, lesions slightly more dorsally in the PVN also lead to hyperphagia and weight gain. Thus a few morphologically well-defined regions of the hypothalamus appear to play a major role in the regulation of body weight and endocrine function. However, rather than specific hypothalamic nuclei controlling energy homeostasis, it is now thought to be regulated by neuronal circuits, which signal using specific neuropeptides (see Fig. 2).

1. ARC

The ARC is thought to play a pivotal role in the integration of signals regulating appetite. The ARC lies in
close proximity to the median eminence, which lacks a complete BBB (51), and thus it is uniquely placed to respond to circulating hormonal signals. Certain plasma hormones, for example, PY and GLP-1, cross the BBB via nonsaturable mechanisms (206, 307). Other signals, such as leptin, are actively transported from blood to brain via saturable mechanisms (28). Thus the BBB may play a dynamic role in regulating the passage of peripheral signals.

Two primary neuronal populations within the ARC integrate signals of nutritional status and influence energy homeostasis (84) (see Fig. 3). A subpopulation of neurons in the medial ARC express the orexigenic neuropeptides NPY and AgRP (53, 166). These neurons project primarily to the ipsilateral PVN (26) but also locally within the ARC. A subpopulation of ARC NPY neurons release GABA locally to inhibit the adjacent POMC neurons. More laterally lies a second subpopulation that inhibits food intake via the expression of CART and POMC, which is processed to α-melanocyte stimulating hormone (α-MSH) (123, 222). This subpopulation projects much more widely within the CNS, to hypothalamic nuclei such as the DMH, LHA, and perifornical area (PFA) as well as the PVN (124, 129, 202). Schwartz et al. (361) proposed a model of appetite regulation whereby arcuate neurons act as the primary hypothalamic site of action of peripheral hormones, such as insulin and leptin. These modulate activity of arcuate neurons, which in turn project to secondary hypothalamic nuclei, for example, the PVN or LHA. Here, the release of further anorectic or orexigenic peptides is modulated to adjust energy intake and expenditure to maintain a stable body weight.

2. PVN

The PVN acts to integrate neuropeptide signals from numerous CNS regions including the ARC and brain stem (350). Microinjection into the PVN of almost all known orexigenic and anorectic signals alters appetite, for example, NPY (230), ghrelin (237), orexin-A (117, 367), CCK (171), leptin (127, 414), and GLP-1 (414). PVN administration of melanocortin agonists potently inhibits food intake (154, 210). Conversely, PVN injection of a melanocortin antagonist stimulates food intake (154). Electrophysiological recordings from PVN neurons have shown ARC neurons expressing POMC potentiate inhibitory GABAergic signaling within the PVN and thus reduce food intake. In contrast, ARC NPY/AgRP neurons inhibit this GABAergic signaling (92) and stimulate food intake.

Recent work suggests that neuropeptides regulating appetite may signal via a common pathway in the PVN involving AMP-activated protein kinase (AMPK). AMPK is a heterodimer consisting of catalytic α-subunits and regulatory β- and γ-subunits. Multiple anorectic signals such as leptin, insulin, and the melanocortin agonist MT-II reduce α2-AMPK activity in the ARC and PVN, whilst orexigenic signals such as AgRP and ghrelin increase α2-AMPK activity (13, 279). Pharmacologically mediated increases in PVN AMPK activity increased food intake (13). Peripherally circulating regulators are unable to modulate α2-AMPK activity in mice lacking the melanocortin 4 receptor (MC4R), suggesting α2-AMPK activity may be controlled by MC4R (279).

Many neuropeptides that modulate appetite also influence endocrine function, for example, thyroid function and hence energy expenditure. The PVN plays a major role in integration of these functions. Both NPY/AgRP and melanocortin projections from the ARC terminate on thyrotropin-releasing hormone (TRH) neurons in the PVN (136, 241). NPY/AgRP inhibits pro-TRH gene expression (137), while α-MSH stimulates pro-TRH expression and inhibits the fasting-induced suppression of TRH (136). The PVN also contains CRH-expressing neurons. NPY projections from the ARC influence CRH expression and release, and this in turn may modulate energy homeostasis (347).

3. DMH

There is evidence for a role of the DMN in the modulation of energy intake. Destruction of the DMN results in hyperphagia and obesity, although less dramatically than VMN lesioning (41). Injection of orexigenic peptides, NPY, galanin, and GABA into the DMN increases food intake (200, 227, 375), and central NPY injection induces c-fos in the DMN (452). The DMH has extensive connections with other hypothalamic nuclei. It receives AgRP/ NPY neurons from the ARC (202) but also contains NPY-expressing cell bodies. α-MSH immunoreactive fibers lie
in close proximity to these NPY-expressing cells, and DMH administration of melanocortin agonists has been demonstrated to reduce both local NPY expression and suckling-induced hyperphagia in rats (71).

4. LHA/PFA

Other hypothalamic areas including the lateral hypothalamic area and perifornical area (LHA/PFA) are involved in downstream signaling. Indeed, the PFA is one of the most sensitive areas for NPY-induced feeding, more so than the PVN (378). The LHA/PFA contains melanin-concentrating hormone (MCH) expressing neurons (266). Here, MCH expression is regulated by nutritional status, since fasting induces MCH mRNA expression. MCH appears to have a powerful role in appetite regulation. Repeated intracerebroventricular injection of MCH increases food intake (327) and adiposity in rats (266). Conversely, MCH-1 receptor antagonists inhibit feeding, and chronic administration leads to a sustained reduction in body weight gain (49). Overexpression of prepro-MCH results in mice that are hyperphagic and centrally obese (266), whereas MCH null mice are lean, hypophagic with increased energy expenditure, despite reduced anorectic signals such as plasma leptin and ARC POMC expression (266, 365). Mice that lack both MCH and leptin have reduced weight gain and adiposity compared with leptin-deficient ob/ob mice (364). This suggests MCH may be a downstream mediator of leptin’s and POMC’s effects on feeding.

The LHA/PFA also contain neurons expressing prepro-orexin and releasing the peptide products orexin A and B (or hypocretin 1 and 2). The orexin-immunoreactive cell population is distinct from that which produces MCH (109, 343). Orexin neurons project widely through the CNS including the PVN, ARC, NTS, and dorsal motor nucleus of the vagus (109, 320) and to areas associated with arousal and attention as well as feeding. Orexin A has high affinity for the orexin-1 receptor, which is highly expressed in the VMH. Orexin A and B have equal affinities for the orexin-2 receptor, and this is expressed primarily within the PVN (343). Prepro-orexin mRNA is upregulated by fasting, and central administration of orexin A results in general arousal and probably a secondary increase in orexigenic behavior (162, 177, 343). However, although central administration of orexin A stimulates daytime feeding, there is no increase in 24-h food intake (177). Furthermore, chronic administration of orexin A does not alter body weight (446). Orexin-knockout mice are thought to be a model of human narcolepsy (68) rather than altered energy balance. However, in circumstances of food deprivation, orexins may mediate both a feeding response and arousal to initiate food-seeking behavior.

It is possible that orexins may also act as peripheral regulators of energy homeostasis. Orexin neurons are found in the gastrointestinal tract. They express both orexin and leptin receptors and appear to be activated by starvation (215). Orexin is also expressed in the gastric, intestinal, and pancreatic endocrine cells (215), and peripheral administration increased plasma insulin levels (308) and decreased circulating glucagon (119).

NPY, AgRP, and α-MSH immunoreactive terminals are extensive in the LHA and are in contact with MCH and orexin-expressing cells (52, 124, 190). Central orexin neurons express both NPY receptors (63) and leptin receptors (190) and hence may be able to integrate their actions. A large number of glucose-sensing neurons are present in the LHA (40), and orexin neurons may play a role in this. Hypoglycemia increases orexin mRNA expression and c-fos in the LHA (61, 292). The mechanisms by which the MCH and orexin neurons influence energy homeostasis remain to be fully elucidated. However, major targets are the endocrine and autonomic nervous system, the cranial nerve motor nuclei, and cortical structures (346).

5. VMH

The VMH has been known to play a role in energy homeostasis for many years, since the finding that bilateral VMH lesions induce hyperphagia and obesity. The VMH receives NPY, AgRP, and α-MSH immunoreactive projections from arcuate neurons and, in turn, VMH neurons project onto both hypothalamic nuclei (e.g., DMH) and brain stem regions (e.g., NTS). VMH expression of neuropeptides is modulated by energy status, with altered NPY expression in obese mice (161) and increased MC4R expression in diet-induced obese rats (195). Brain-derived neurotrophic factor (BDNF) is highly expressed in the VMN, and its expression is regulated both by food deprivation and melanocortin agonists (444). Mice with reduced BDNF receptor expression or reduced BDNF signaling have increased food intake and body weight (335, 444). Therefore, BDNF neurons in the VMH may act as an additional downstream pathway through which nutritional status and the melanocortin system modulate energy homeostasis.

B. Hypothalamic Regulators of Appetite

1. NPY

NPY is one of the most abundant neurotransmitters in the CNS (10), but the ARC is the major hypothalamic site of NPY expression (293). Hypothalamic levels of NPY reflect the body’s nutritional status with hypothalamic NPY mRNA and NPY release increasing with fasting and decreasing after refeeding (203, 344, 388). NPY is the most potent orexigen known, and repeated third ventricle or
PVN injection of NPY causes marked hyperphagia and obesity (377, 455). Central administration of NPY also inhibits brown fat thermogenesis (46), suppresses sympathetic nerve activity (118), and inhibits the thyroid axis (137) to reduce energy expenditure. In addition, NPY stimulates basal plasma insulin (282, 455) and morning plasma cortisol (455), effects which are independent of increased food intake.

Despite the potency of NPY’s actions on food intake, NPY null mice have normal body weight and adiposity (396), with the only demonstrable abnormality of energy homeostasis being a reduction in fasting-induced feeding (29). This normal phenotype may be due to the presence of compensatory mechanisms or redundancy in orexigenic pathways, such as those which signal via AgRP (352). In addition, Y5 receptor-deficient mice have an attenuated feeding response to NPY (205). However, contrary to expectation, hypothalamic Y5 receptor density is reduced by fasting and increased by dietary-induced obesity (429). Furthermore, antagonists to the Y5 receptor do not significantly alter food intake in rats (405), and Y5 receptor-deficient mice demonstrate late-onset obesity rather than weight loss (264). It has been suggested that the role of the Y5 receptor is to maintain rather than initiate the feeding response to NPY. This is supported by the observation that Y5 receptor antisense decreases food intake only 10 h after the onset of NPY- or PP-induced feeding and has no effect on the initial orexigenic response (140). Similarly, there is evidence for a role of the Y1 receptor. Y1 receptor antagonists block both NPY- and fasting-induced feeding (204, 430), and Y1 receptor-deficient mice have an attenuated feeding response to NPY (205). However, similarly to Y5 receptors, ARC Y1 receptor density, distribution, and expression are reduced by fasting, and these changes are moderated by glucose administration (72). NPY fragments with poor Y1 binding still increase food intake to the same extent as equimolar doses of NPY (313), and Y1 receptor-deficient mice are obese but not hyperphagic (226). These data suggest the Y1 receptor is not responsible for the NPY feeding effect but may play a role in energy expenditure (226). There is also some support for a role of Y4 receptors in the orexigenic NPY response. PP has a relative specificity for the Y4 receptor, and central administration has been shown to elicit food intake in both mice (19) and rats (63). Y2 and Y4 receptors lie presynaptically and have an autoinhibitory effect on NPY neurons (212, 213). As expected, Y2 receptor null mice are hyperphagic and obese and have increased adiposity (304). However, mice with a conditional knockout of the Y2 receptor, and thus perhaps with more normal neuronal circuitry, have a temporary reduction in body weight and food intake, which returns to normal after a few weeks (340). Thus it is probable that the effects of NPY on feeding are mediated by a combination of receptors rather than a single subtype.

2. Melanocortin system

The melanocortin system is comprised of the peptide products of POMC cleavage, their receptors, and the endogenous melanocortin antagonists AgRP and agouti. Hypothalamic POMC mRNA expression is regulated by nutritional status with low levels in fasting that are restored by exogenous leptin administration or 6 h after refeeding (359, 388). Human POMC gene mutations or abnormal POMC peptide processing result in early-onset obesity and red hair secondary to lack of α-MSH, along with adrenal insufficiency due to loss of ACTH (223). Haploinsufficiency of the POMC gene is sufficient to render mice susceptible to diet-induced obesity (65).

Five melanocortin receptors have been identified, MC1R-MC5R; however, MC3R and MC4R are likely to play a role in energy homeostasis. They are widely expressed in the hypothalamus and are found in the ARC, VMH, and PVN (175, 294). Absence of MC4R results in hyperphagia and obesity in rodents (131, 196), and abnormalities of this receptor have been implicated in 1–6% of severe early-onset human obesity (133, 253, 254). In addition, polymorphism of this receptor has been implicated in polygenic late-onset obesity in humans (15).

Although MC4R involvement in regulation of feeding is well established, the role of MC3R remains unclear. Relatively selective MC3R agonists do not alter food intake (1), and unlike MC4R expression, which is influenced by energy status, MC3R expression is not (175). However, the MC3R/MC4R antagonist AgRP is reported to increase food intake in MC4R null mice (59). In addition, mice lacking MC3R have increased adiposity, although not body weight, and preferentially metabolize carbohydrate rather than fat (60). On high-fat chow, MC3R null mice develop obesity and have a further increase in adipose tissue compared with wild-type littermates. Furthermore, MC3R mutations have been reported in morbidly obese human subjects (283).

The main endogenous ligand for the MC3R/MC4R is α-MSH, which is expressed by cells in the lateral part of the arcuate nucleus (423) (see above). Central administration of MC4R agonists suppresses food intake, while
administration of antagonists results in hyperphagia (38). In addition to its actions on feeding, α-MSH also increases oxygen consumption (321), suggesting increased energy expenditure. α-MSH activates the thyroid axis (211), sympathetic nervous activity, and brown adipose tissue (450).

Two endogenous antagonists of melanocortin receptors have been described: agouti and AgRP. The agouti protein is a competitive antagonist of α-MSH at MC1R and MC4R (252). Agouti expression is normally restricted to the hair follicle where its antagonist effect on the peripheral MC1 receptor results in a yellow pigment. However, the agouti mouse ectopically expresses the agouti protein within the CNS, thereby antagonizing the actions of α-MSH at the hypothalamic MC4R resulting in hyperphagia and obesity (131, 252). Unlike agouti, AgRP is expressed in the CNS, primarily in the medial part of the arcuate nucleus (370). AgRP is partially homologous to agouti peptide and is a potent selective antagonist at MC3R and MC4R (311). AgRP mRNA expression is increased by fasting, and unlike NPY mRNA levels, which are reduced 6 h after refeeding, AgRP levels remain elevated (388). Central administration of AgRP or AgRP-(83—132), the carboxy-terminal fragment, is able to block α-MSH-induced anorexia and increase nocturnal food intake (337). Moreover, this hyperphagia has been reported to persist for up to a week after a single injection (165, 337). This prolonged response results in a greater cumulative effect on food intake than NPY, and probably involves more diverse signaling pathways than the melanocortin pathway alone (164, 165, 459). Independent of its effects on food intake, AgRP may increase body weight via decreased energy expenditure. Repeated central administration of AgRP suppresses TRH, reduces oxygen consumption, and decreases the ability of brown adipose tissue to expend energy (372, 373). Transgenic mice overexpressing AgRP are obese but, as AgRP is inactive at the MC1 receptor results in a yellow pigment. However, the hair follicle where its antagonist effect on the peripheral MC1 receptor results in a yellow pigment. However, the agouti mouse ectopically expresses the agouti protein within the CNS, thereby antagonizing the actions of α-MSH at the hypothalamic MC4R resulting in hyperphagia and obesity (131, 252).

AgRP and NPY are colocalized in 90% of ARC neurons (53, 166). Activation of ARC NPY/AgRP neurons potently stimulates feeding via a number of pathways: the orexigenic effect of NPY released in the PVN, AgRP antagonism of MC3R/MC4R in the PVN, and local release of NPY and GABA within the ARC to inhibit the arcuate POMC neurons via Y1 and GABA receptors, respectively (149, 336). However, NPY/AgRP knock-out mice have no obvious feeding or body weight defects, and AgRP is not present in other hypothalamic nuclei known to be involved in energy homeostasis, such as the VMH (53). Therefore, there must be other signaling pathways regulating energy homeostasis (326).

3. CART

CART is the third most abundant transcript within the hypothalamus and is expressed in the ARC (123, 222) (with POMC, LHA, and PVN (91). Food deprivation reduces ARC expression of CART, whereas peripheral leptin replacement to ob/ob mice stimulates CART expression (222). CART-(1—102) and CART-(82—103) injected into the third cerebral ventricle inhibit both normal and NPY-stimulated feeding in rats, but also cause abnormal behavioral responses at high doses (222, 229). Intracerebroventricular injection of antiserum against CART peptide-(1—102) and CART peptide fragment-(82—103) increases nocturnal feeding, suggesting CART is a physiological regulator of energy homeostasis (222, 229). However, injection of CART-(55—102) into discrete hypothalamic nuclei such as the ARC and VMN actually increases food intake (2). Thus there may be several populations of CART-expressing neurons with differing roles in feeding. For example, NPY release could stimulate a population of CART neurons in the ARC that are orexigenic, producing positive orexigenic feedback (111).

C. Reward and Regulation of Appetite

Even in the absence of an energy deficit, the rewarding nature of food may act as a stimulus to feeding. However, there is interaction between nutritional status and the sensation of reward, as the subjective palatability of food differs between the fed and fasting states (43). Signals of energy status, such as leptin, are able to modulate reward pathways (148).

1. Opioids

The reward circuitry is complex, involving interactions between several signaling systems, including opioid, dopaminergic, and cannabinoid systems. Opioids play an important role. Mice lacking either enkephalin or β-endorphin lose the reinforcing property of food, regardless of the palatability of the food tested. However, the reinforcing effect is regained in fasted animals; thus homeostatic mechanisms can override the hedonistic pathways (178). In humans, opiate antagonists reduce food palatability but without altering subjective hunger (114, 451). The nucleus accumbens (NAC) forms an important part of the reward circuit. Microinjection of opioid agonists into the NAc stimulates CART expression of CART, whereas peripheral leptin replacement to ob/ob mice stimulates CART expression (222). CART-(1—102) and CART-(82—103) injected into the third cerebral ventricle inhibit both normal and NPY-stimulated feeding in rats, but also cause abnormal behavioral responses at high doses (222, 229).

2. Endocannabinoids

The appetite-stimulating effects of marijuana (Cannabis sativa) have been known for a long time (3). The
discovery of cannabinoid receptor type 1 (CB1) and cannabino
doid receptor type 2 (CB2) (110, 269, 295), as well as the
characterization of endogenous ligands for these recep-
tors, the endocannabinoids, have prompted further
investigation of this system. Several studies have indi-
cated that administration of cannabinoids stimulates food
intake in animal models (218, 433). Appetite is increased
by both peripheral and central administration of anand-
amide, one of the major endocannabinoids, in rodents
(173, 200, 432). This orexigenic effect may be mediated
via CB1 receptors in the hypothalamus, which colocalize
with CART, MCH, and orexin peptides (90). A CB1 recep-
tor antagonist has been shown to reduce food intake (81),
and CB1 knock-out (CB1−/−) mice show reduced caloric
intake and decreased body weight (90). However, CB1−/−
mice pups are able to overcome initial absence of milk
ingestion, suggesting development of compensatory
mechanisms that may involve an additional CB3 receptor
(144). Defective leptin signaling is associated with high
hypothalamic endocannabinoid levels in animal models
(112). A recent study shows a synergistic interaction be-
tween the cannabinoid and melanocortin systems in reg-
ulating food intake (418). It also suggests that the canna-
bainoid receptors are located downstream from the mel-
acortin system and that activation of CB1 receptors is
necessary to prevent the melanocortin system from alter-
ing food intake (418). Interestingly, CB1 receptors are
also present on adipocytes where they appear to act
directly to increase lipogenesis (90). There is currently a
CB1 selective antagonist, Rimonabant, in phase 3 clinical
trials that may be a potentially promising antiobesity
drug.

3. Others

The dopaminergic system is also integral to reward-
induced feeding behavior. The effects of central dopa-
mine signaling on feeding are thought to be mediated by
D1 and D2 receptors (225, 354). Mice that lack the tyrosine
hydroxylase gene and therefore dopamine have fatal hy-
pophagia. Tyrosine hydroxylase gene replacement, and
hence dopamine replacement, into the caudate putamen
restores feeding, while gene therapy into either the cau-
date putamen or NAc restores preference for a palatable
diet (389).

Reciprocal GABAergic connections exist between
the NAc and LHA, and it is possible that disinhibition of
LHA neurons may mediate hedonistic feeding (381). The
LHA may also reciprocally influence reward circuits via
MCH expressing neuronal projections as MCH receptors
are expressed in the NAc (341).

Other systems, including those mediated by seroto-
nin, may also be able to modulate both reward circuitry
and homeostatic mechanisms controlling feeding. Seroto-
nin may directly influence the melanocortin pathway in

the ARC via 5-hydroxytryptamine (5-HT) receptors (179).
The now-discontinued anorectic agent fenfluramine me-
diates its actions via 5-HT (170). Fenfluramine acts via
two mechanisms to increase 5-HT release. First, it binds
to 5-HT transporter proteins that move the drug into the
nerve terminal in exchange for 5-HT which moves into the
synapse, and second, it is a substrate for the vesicular
monoamine transporter that disrupts the compartmental-
ization of 5-HT in vesicles and increases the cytoplasmic
pool of 5-HT available for release.

The noradrenergic system also plays a role in appe-
tite regulation, with activation of α1- and β2-adrenergic
receptors inhibiting food intake. Phentermine acts as a
norepinephrine reuptake inhibitor, thereby increasing
synaptic norepinephrine to reduce appetite and weight
gain (35). In contrast, activation of α2-adrenergic recep-
tors increases food intake.

Neurotensin, a 13-amino acid peptide with neurons
and terminals in hypothalamic areas including the ARC
and PVN (197), has also been shown to decrease food
intake when administered centrally (245). Expression of
neurotensin is downregulated in the ob/ob mouse (431).
Studies also suggest that neurotensin mediates the central
effect of leptin on food intake (339).

D. Brain Stem Regulators of Appetite

Extensive reciprocal connections exist between the
hypothalamus and brain stem, particularly the NTS (333,
395, 413). The brain stem plays an important role in the
regulation of energy balance. The NTS is in close anatomical
proximity to the area postrema, a circumventricular
organ with an incomplete BBB (125). Like the ARC, the
NTS is therefore in an ideal position to respond to periph-
eral circulating signals but in addition also receives vagal
afferents from the gastrointestinal tract and afferents
from the glossopharyngeal nerves (201, 349).

1. GLP-1

The NTS contains NPY, melanocortin, and GLP-1
neuronal circuits. GLP-1 forms the major brain stem cir-
cuit regulating energy homeostasis. In the CNS, GLP-1 is
synthesized exclusively in the caudal NTS, and these pre-
proglucagon neurons also express leptin receptors. GLP-1
immunoreactive fibers then project widely, but particu-
larly to the PVN and DMN, with fewer projections to the
ARC. GLP-1 receptor expression is also widespread, both
within the hypothalamus (PVN, DMH, and supraoptic nu-
cleus) and in the brain stem (subfornical organ, organum
vasculosum laminae terminalis, and area postrema). Cen-
tral administration of GLP-1, either into the third or fourth
ventricle, potently reduces fasting and NPY-induced food
intake (406), and blockade of endogenous GLP-1 with the
GLP-1 receptor antagonist exendin-(9—39) increased
food intake (406). This suggested a role of endogenous hypothalamic GLP-1 in energy homeostasis. The anorectic effect of GLP-1 is completely abolished in animals treated with MSG; thus the ARC appears to be vital for GLP-1’s anorectic action (391). There is still debate about the role of conditioned taste aversion (CTA) in the reduced food intake seen following central GLP-1 administration. However, Kinzig et al. (214) have dissociated the anorectic actions of GLP-1 from the induction of CTA following fourth ventricle injection (214). Data regarding the long-term effects of central GLP-1 are also conflicting. Continuous infusion of GLP-1 was initially reported not to alter food intake or body weight (113) but later studies with either continuous central administration or repeated intracerebroventricular injection reduced both (107, 273). However, mice lacking the GLP-1 receptor do not show any abnormality of food intake or body weight (362).

2. Others

NPY neurons from the brain stem project forward to the PVN (351), and extracellular NPY levels within the NTS are modulated by feeding (453). A high density of NPY binding sites, including Y1 receptors and Y5 receptors, are found in the NTS (115, 156, 174). There is also evidence for a separate melanocortin system in the NTS (208). POMC-derived peptides are synthesized in the NTS of the rat (55, 141, 208) and caudal medulla of humans (159). Brain stem POMC neurons are activated in response to food intake and also by CCK administration (131). MC4R are also expressed in the NTS (294) and act to regulate energy intake. Fourth ventricle injection of a MC3R/MC4R agonist or administration into the dorsal motor nucleus of the vagus nerve reduces food intake. Conversely, MC3/4 receptor antagonist administration to these areas increase intake (434).

Prolactin-releasing peptide (PrRP), the endogenous ligand of the previously orphan G-coupled receptor GPR10, is expressed in the NTS in addition to the hypothalamic DMH (240). PrRP neurons are reduced in fasting rats, and third ventricle administration of PrRP or injection into the DMH decreases nocturnal and fasting-induced food intake (363). These effects may be mediated by CRH (236). In addition, peripheral administration of CCK activates brain stem PrRP neurons, suggesting it mediates CCK’s central actions (235). However, repeated administration of PrRP did not alter food intake after the initial 72 h (126) and therefore may play a role in short-term appetite regulation rather than in control of body weight in the longer term.

IV. FUTURE DIRECTIONS

Long-term signals of energy stores and short-term fluctuations in food intake are released from adipose tissue and the gut endocrine system. These signals are integrated in the hypothalamus and brain stem. Important neuropeptide signals such as NPY, AgRP, and the melanocortins are released and influence activity of diverse circuits within other hypothalamic nuclei, which signal using a wide range of transmitter systems (Fig. 4). This homeostatic process results in subsequent changes in appetite, behavior, and energy expenditure.

The recent clarification of the function of gut hormones, adiposity signals, and hypothalamic neurotransmitters has greatly expanded our understanding of the physiology of energy balance. Although this system is designed to maintain body weight, complex interactions between genetic and environmental factors may impinge on both peripheral signals and central pathways to result in obesity. The increasing global prevalence of obesity...
makes understanding these factors an important priority. A more detailed understanding of the pathogenesis of obesity may make successful treatment possible.

New approaches could be tailored to provide an effective solution for the individual. Leptin replacement has successfully treated the uncommon form of obesity due to leptin deficiency. Similarly, obesity that results, for example, from reduced melanocortin signaling in the brain may respond to a melanocortin receptor agonist. Individually tailored therapy or combination therapy will be more effective than the currently available pharmacological agents that are of limited efficacy and duration (see review in Ref. 138).

Mimicking postprandial satiety by modulation of circulating gastrointestinal hormones may provide a possible means of treating obesity. Interestingly, the reduction in weight and appetite (22) seen in subjects following gastrointestinal bypass surgery may be the result of altered gastrointestinal hormone release, for example, elevated PYY and OXM (302, 348) and/or suppressed ghrelin levels (99). Although surgery is an effective long-term treatment for obesity, it is a major operation with significant associated mortality, and so is rightly restricted to those with severe morbidity obesity. The changes in circulating gut hormones secondary to gastrointestinal bypass surgery suggest that modulation of gut hormones, by other means, may be an effective long-term therapy for obese individuals. In addition, in contrast to drugs which affect widely distributed central neurotransmitters or their receptors, modulation of peripheral signals would target regions of the brain controlling appetite more specifically.

Efforts to develop pharmacological treatments for obesity have multiplied over the last decade, and a number of therapies are currently being investigated in phase II and III clinical trials. Attention is turning to the hedonic aspects of food intake with the development of endocannabinoid antagonists. A fuller understanding of the regulation of food intake will hopefully allow the rational development of drugs that are able to reverse the ongoing acceleration of the current obesity epidemic.

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