Lori Asarian* and Thomas Bächler

Neuroendocrine control of satiation

Abstract: Eating is a simple behavior with complex functions. The unconscious neuroendocrine process that stops eating and brings a meal to its end is called satiation. Energy homeostasis is mediated accomplished through the control of meal size via satiation. It involves neural integrations of phasic negative-feedback signals related to ingested food and tonic signals, such as those related to adipose tissue mass. Energy homeostasis is accomplished through adjustments in meal size brought about by changes in these satiation signals. The best understood meal-derived satiation signals arise from gastrointestinal nutrient sensing. Gastrointestinal hormones secreted during the meal, including cholecystokinin, glucagon-like peptide 1, and PYY, mediate most of these. Other physiological signals arise from activation of metabolic-sensing neurons, mainly in the hypothalamus and caudal brainstem. We review both classes of satiation signal and their integration in the brain, including their processing by melanocortin, neuropeptide Y/agouti-related peptide, serotonin, noradrenaline, and oxytocin neurons. Our review is not comprehensive; rather, we discuss only what we consider the best-understood mechanisms of satiation, with a special focus on normally operating physiological mechanisms.

Keywords: brain; eating; gastrointestinal; gut peptides; nutrient sensing.

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Introduction

Energy homeostasis refers to two processes: (a) the regulation of an adequate readily available supply of energy metabolites in the circulation and (b) the regulation of adipose tissue mass, which is the major energy store. These are among the most important biological functions of any organism. Both aspects of energy homeostasis are linked to eating. Eating normally results in the entry of sufficient energy metabolites from the intestines into the circulation except during intense exercise and the "postabsorptive state", i.e., early in the morning, when the stomach is entirely empty and intestinal absorption slows. Glycogenolysis and lipolysis take up the slack in such situations. Over the longer term, stability of the adipose tissue mass, or "lipostasis", requires that the energetic content of food eaten be balanced with energy expended in metabolism and physical work (in adults, in whom growth can be ignored). If this energy equation is imbalanced, fat stores and body weight will drift upward or downward. Perhaps the most convincing evidence that adipose tissue mass is actively regulated is that experimental perturbations of adipose tissue mass lead to compensatory eating and energy expenditure responses that re-establish normality [1]. Figure 1 shows an example [2, 3].

Humans, rats, and mice eat in the form of meals. Thus, study of the neuroendocrine bases of eating should, and ultimately must, target the mechanisms controlling meal size and frequency. Furthermore, the homeostatic controls of eating described above must be manifested as changes in patterns of meal frequency or meal size. Meal size is the clearly decisive parameter in rats: (a) the total amount eaten over days correlates with average meal size but not with meal frequency [4]; (b) diluting the energy content of the diet results in immediate increases in meal size, with little change in meal frequency [5]; (c) in a cold environment, rats ate larger, but less frequent, meals [6]; (d) diabetic rats that cannot metabolize glucose normally increase meal size rather than meal frequency [5, 7, 8]; (e) during lactation, rats increase meal size sooner and more than meal frequency [9]; (f) the increased energetic demand brought about by female rats' avid wheel running is met by increasing meal size [10]; (g) the hyperphagia of obese Zucker rats, which have defective leptin receptor signaling, is caused by increased meal size rather than meal frequency [11, 12], and chronic leptin administration increases meal size, not meal frequency [13, 14], and perhaps most convincing, (h) compensatory eating in rats recovering from enforced overweight or forced underweight is usually accomplished by adjusting meal size, not meal frequency [2, 3, 15–17] (Table 1). In contrast to the selective effect of energy-homeostatic challenges on meal

***Corresponding author: Lori Asarian,** Institute of Veterinary Physiology, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland, Phone: +41 44 635 8836, Fax: +41 44 635 8932, E-mail: lasarian@vetphys.uzh.ch
Lori Asarian: Center of Integrative and Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland
Thomas Bächler: Institute of Veterinary Physiology, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland

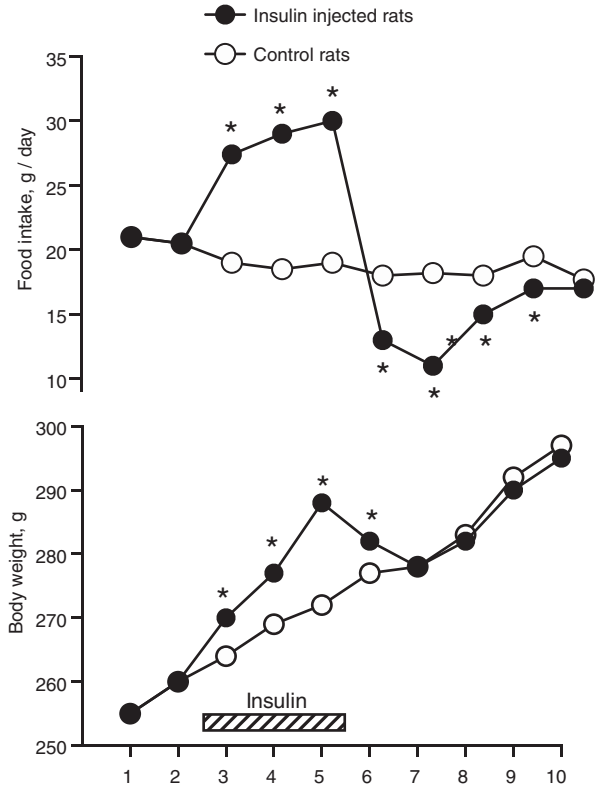

Figure 1 Changes in food intake (top) and body weight (bottom) during and after insulin injections. Filled circles, injected animals; open circles, control animals; shaded bar, injection period. *Different from controls.

Table 1 Food intake and meal patterns during postinsulin hypophagia.

	Baseline level		Postinsulin hypophagia	
	Diurnal	Nocturnal	Diurnal	Nocturnal
Food intake, g/12 h	7.2±0.5	18.5±0.4	5.6±0.5[a]	12.8±0.5[a]
No. of meals	4.3±0.3	8.6±0.4	3.7±0.2	7.5±0.5
Meal duration, min	13.5±0.8	17.2±1.0	10.2±0.8[b]	13.0±1.0[a]

Values are mean±SE for 11 rats. Baselines are the rats' mean values during 4 days before insulin treatment. Hypophagic values were measured on the second day after the end of insulin treatment. [a]Hypophagic value different from baseline, paired t-test, $2\alpha<0.05$. [b]$p<0.01$.

size, rats' meal frequency seems more sensitive to the very different homeostatic challenges such as those occurring during illness [18–21]. Furthermore, in humans, social conditioning is normally the major controller of meal timing. For these reasons, we focus here on the mechanisms by which energy homeostasis controls meal size.

Current understanding indicates that the contributions of meal size to the two aspects of energy homeostasis described above arise from two different classes of feedback signals. The first class, described by Smith [22] as the direct controls of meal size, consists of negative feedback that are generated during meals in the gastrointestinal tract from preabsorptive nutrient stimuli, and their primary effect is to inhibit eating and bring the meal to an end, i.e., to produce satiation. This feedback mechanism is shown schematically in the inner feedback loop in Figure 2. We will discuss the gastrointestinal nutrient-sensing mechanisms mediating the direct controls of satiation below. The second class of feedback comprises circulating energy metabolites and hormones involved in energy metabolism. These arise from nutrient absorption and from organs involved in energy metabolism such as the liver, pancreas, and adipose tissue, and they act on a widely distributed set of what Levin and colleagues [23, 24] call "metabolic-sensing neurons". Lipostasis is one of the important functions of this feedback which is shown schematically in the outer feedback loop in Figure 2. Finally, we will discuss the central neural networks that integrate this information and produce satiation. An important point reflected in Figure 2 is that current understanding indicates that the gastrointestinal nutrient- and metabolic-sensing neural networks converge in the caudal brainstem, which also organizes the motor control of satiation. Note that this current understanding indicates that this indirect feedback loop controls meal size by converging on the central neural networks mediating the direct control of satiation.

Figure 2 also shows several other controls of meal size that we do not include in this review. For example,

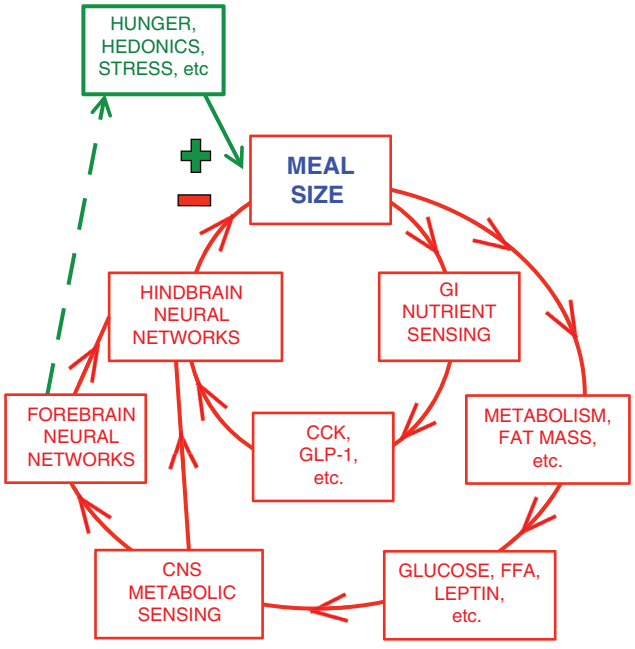

Figure 2 Diagram of proposed meal size controls.

although "hunger" mechanisms such as ghrelin contribute primarily to meal initiation, they also affect meal size [25]. Their contribution to homeostatic eating, however, is less clear. "Hedonics" refers to the pleasurable aspects of orosensory food stimuli. Food hedonics can tonically increase meal size and lead to increased adiposity [26–28]. In the modern Western "obesogenic" environment [27, 28], these effects more often detract from homeostasis than contribute to it. The same is true of several stressors that primarily affect meal size [25, 29, 30].

Our review is not comprehensive; rather, we review only what we consider are presently the best-understood mechanisms of satiation. Finally, we also emphasize that most, probably all, of the physiological controls of meal size that we review are powerfully modulated, first, by sex and, second, by experience. We do not review these topics; the interested reader is referred to [31] and [32–36], respectively.

Gastrointestinal nutrient sensing and satiation

Which gastrointestinal nutrient-sensing mechanisms contribute to satiation?

In this section, we review the molecular gastrointestinal nutrient-sensing mechanisms and gastrointestinal hormone-signaling mechanisms involved in satiation. Gastrointestinal nutrient sensors contribute to the control of eating through three mechanisms. First, they initiate neural signaling via vagal and spinal visceral afferent nerves. The vagus in particular is a major route of information between the gut and the brain. Vagal afferents project to the nucleus of the solitary tract (NTS) and, via the NTS, throughout the dorsal vagal complex (DVC) of the hindbrain, an important node in the central neural network controlling eating. Second, gastrointestinal nutrient sensors stimulate enteroendocrine cells in the gastrointestinal mucosa to release a variety of hormones with roles in digestion, metabolism, and the control of eating. Our review concentrates on three hormones with putative roles in satiation: cholecystokinin (CCK; secreted by I-cells), glucagon-like peptide 1 (GLP-1; secreted by L-cells), and peptide YY (PYY; secreted by L-cells).

The apical surfaces of the enteroendocrine cells contact the gut lumen, whereas the basolateral surface contacts the lamina propria, in close proximity to the circulatory and lymphatic systems. Because of this "open-type" morphology, these cells are in perfect position to sense incoming nutrients as they arrive in the gut lumen, perform intracellular integratory operations, and generate a response in the form of hormone secretion. As in other endocrine cell types, alterations in membrane potential are coupled via voltage-gated Ca^{2+} entry to the release of secretory vesicles. All enteroendocrine cell types studied to date are also responsive to signals that activate G-protein-coupled receptors (GPCRs). Finally, gastrointestinal nutrient sensors affect the enteric nervous system. Enteric nervous system reflexes, often involving gastrointestinal hormones, regulate gastrointestinal nutrient flow during and after eating by mixing, propelling, and separating luminal contents. The regulation of gastric emptying is an especially important function that indirectly affects eating.

Gastrointestinal carbohydrate sensing

A variety of pathways have been implicated in the physiological responses of the gut to carbohydrates. The most information concerns glucose transporters, which can be coupled to Na^+ (e.g., SGLT1) or facilitative transporters (e.g., GLUT1, GLUT2, GLUT5). SGLT1 on the apical surface of enteroendocrine cells [37, 38] contributes to glucose absorption via Na^+-dependent active transport. The Na^+ gradient is maintained by the sodium-potassium pump located on the basolateral membrane surface. Glucose uptake is therefore a secondary active-transport system in which ATPase indirectly provides the energy. SGLT1 mRNA is increased by high-carbohydrate meals [39]. SGLT upregulation was abolished in rats following chemical vagal deafferentation [40], suggesting a role of the vagus in carbohydrate uptake by enteroendocrine cells.

The release of GLP-1 from L-cells and glucose-dependent insulinotropic peptide (GIP) from K cells is stimulated by the activation of SGLT1, as indicated by its blockade by the inhibitor of sodium-coupled uptake, phloridzin [41, 42]. Interestingly, GIP secretion requires nutrient absorption, whereas GLP-1 secretion is triggered just by presence of nutrients in the lumen.

SGLT1 mRNA is found at relatively high levels in K- and L-cells in both the small intestine and colon [43, 44] and in the mouse colon [45]. SGLT3 has been implicated in glucose-triggered serotonin (5HT) release from rat enterochromaffin cells and downstream activation of vagal afferents via 5HT-3 receptors [46, 47]. Thus, using

phosphorylated Ca/CAMKII (pCAMKII) as a marker of cells activated by elevated Ca^{2+}, Vincent et al. [48] found that pCAMKII was detectable in rat enterochromaffin cells and in vagal-afferent cell bodies following luminal application of glucose, but not galactose. However, a full characterization of the substrate preferences of mouse SGLT3a and the rat SGLT3 isoforms is clearly required before it will be possible to use substrate and inhibitor pharmacology as reliable indicators of rodent SGLT3 activation.

Dietary fructose stimulates GLP-1 secretion. Apical fructose transport in the intestine occurs via the facilitative sugar transporter GLUT5, whose activity is not electrogenic and has not been linked directly to activation of intracellular signaling pathways. Thus, the molecular identity of the GLUT5 sensor and how fructose triggers GLP-1 release in vivo remains obscure. L-cells express high levels of GLUT5 mRNA [44], suggesting they can take up fructose from the intestinal lumen. However, although absorbed fructose could enter the metabolic pathway, with consequent generation of ATP and closure of K_{ATP} channels, it seems unlikely that this accounts for fructose-triggered GLP-1 release in vivo because the simple closure of K_{ATP} channels by sulphonylureas is not a sufficient trigger for secretion [49]. Perhaps the enhanced metabolic rate may generate additional signals, including ATP itself, that stimulate GLP-1 secretion.

The sweet-taste receptors α-gustducin and T1R2/T1R3 also contribute to glucose sensing in enteroendocrine cells, particularly K- and L-cells. α-Gustducin knockout (KO) mice showed reduced levels of both GLP-1 and GIP compared with wild-type mice [50]. However, expression profiling of purified K- and L-cells revealed no enrichment of mRNA for T1R2/T1R3 or α-gustducin in response to glucose [43, 44]. In humans, however, intragastric infusion of the T1R2/T1R3 sweet-receptor inhibitor lactisole reduced the GLP-1 response to glucose [51, 52], indicating that T1R2/T1R3 sweet receptors mediate part of the direct effect of carbohydrates on GLP-1 secretion. Clearly, further work is required to clarify the roles of sweet-taste receptor in gastrointestinal hormone secretion.

The last step of absorption, basolateral glucose efflux, is facilitated by GLUT2 in the proximal gastrointestinal tract and by GLUT1 more distally [45]. GLUT2 is transiently recruited to the apical-membrane surface after meals, allowing passive absorption of glucose at higher luminal glucose concentrations. Mace et al. [53] reported that sucralose doubled the rate of glucose absorption by increasing the expression of GLUT2. T1R2/T1R3 can act as a glucose sensor at high concentrations and may control the apical expression of GLUT2 and consequent increased capacity to absorb glucose.

Gastrointestinal lipid sensing

Ingested lipids are potent stimuli for the secretions of CCK, GIP, GLP-1, and PYY. Although enteroendocrine cells may sense triglycerides, most evidence indicates that prior lipolysis is required [54]. Long-chain fatty acids (LCFA; 12 carbons or more) are potent CCK secretagogues [55, 56]. The vagal stimulation that is required for these responses by LCFA has been attributed to an indirect process, whereby the free fatty acids (FFAs) are sensed by I cells and the release of CCK acts in a paracrine manner to stimulate CCK-1 receptors on the vagal afferents. For lipid-induced CCK release, chylomicron formation is essential for this process. Pluronic L-81 or specific inhibitors of microsomal triglyceride-transfer protein inhibit chylomicron formation and reduce CCK secretion [56]. Because enteroendocrine cells cannot produce chylomicrons, it is possible that neighboring enterocytes serve as lipid sensors. Chylomicron synthesis does not seem to play a role in fat-triggered GLP-1 and PYY release.

All FFA receptors (FFAR1–3, GPR120) are GPCRs [57–60], and enteroendocrine cells express their mRNA [43, 44, 61]. Medium-chain fatty acids and LCFAs activate FFAR1 (GPR40) and GPR120, which stimulate the Gq-phosphatidylinositol pathway, increasing Ca^{2+} concentrations and exocytosis [57, 59]. Knocking out FFAR1 in mice decreases high-fat diet-induced GLP-1 and GIP release [62] and impaired linoleic-acid-stimulated CCK secretion [63]. In vitro siRNA knockdown of GPR120 reduced both GLP-1 and CCK secretion [59, 64].

FFAR2 (GPR43) and FFAR3 (GPR41) are present in the distal intestine, especially the colon. Small-chain fatty acids, which can activate GPR43 via either the Gq-phosphatidylinositol or the Gi/o pathways or GPR41 via the Gi/o pathway [60], are involved in colonic release of GLP-1.

GPR119 is in a special category because lipids do not activate it, but oleoylethanolamine (OEA) does [65]. GPR119 is present in L- and K-cells [43, 44] and participates in GLP-1, PYY, and GIP release [66]. Ingestion of nutrients stimulates the release of OEA in the small intestine, which can induce the release of GLP-1 [67].

Finally, CD36 is a transporter for FFAs that was initially described in taste papillae [68–71]. It is believed to act via Src-PTK-B, PLCβ2, and TRPM5 and as a co-receptor for GPR120 [72]. CD36 is expressed in the brush border of duodenal and jejunal epithelia. CD36-KO mice have reduced OEA production and OEA-induced satiety [73],

the latter of which can be induced via PPARα activation [73, 74].

Gastrointestinal protein and amino acid sensing

The most information on protein sensing relates to CCK secretion. In vitro experiments using the STC-1 cell line indicate that CCK expression in response to peptones is mediated via GPR93 [75]. Although it has been shown that GPR93 recruits Gq-, Gs-, Gi-, and G12/13-mediated signaling pathways [76–78], its role in physiological peptone sensing is still under investigation. L-Glutamate seems to act via the heterotrimeric T1R1/T1R3 receptor [79, 80], which is expressed in enteroendocrine cells [81]. L-Phenylalanine couples with the CaSR receptor to activate a phosphatidylinositol pathway, which results in Ca^{2+}-induced release of hormones including CCK [63, 82, 83].

In vitro studies using the GLUTag, NCI-H716, and STC-1 cell lines show that peptide transporters such as PepT1 (SLC15A1) and PepT2 (SLC15A2) are activated by protein hydrolysates, resulting in increased intracellular Ca^{2+} and hormone release. Glutamine stimulation, for example, leads to the release of GLP-1 from GLUTag and primary colonic cultures.

What neuroendocrine mechanisms mediate gastrointestinal satiation?

Vagus nerve

The importance of vagal afferents in satiation is reflected in the increase in meal size that follows selective surgical section of sensory abdominal vagal afferents. Considerable effort has been devoted to determining the brainstem organization of gastrointestinal vagal afferent projections. The NTS is the first synaptic site for gastrointestinal afferents of the IX and X cranial nerves (i.e., glossopharyngeal and vagus nerves, respectively). Reconstruction of these projections in the horizontal plane reveals a viscerotopic map, with the rostrocaudal distribution of terminal fields corresponding to their rostrocaudal origins in the gastrointestinal tract. Furthermore, gastric and small-intestinal afferents project mainly to the commissural and medial nuclei of the NTS. In addition, several brain areas including the medial frontal cortex, insular cortex, central nucleus of the amygdala, paraventricular nucleus of the hypothalamus, lateral hypothalamic area, and others can influence the output of the NTS by virtue of their connectivity with the dorsal vagal complex and thus be involved in the control of normal digestive processes.

The dorsal motor nucleus of the vagus (DMX) is the major source of visceral efferents that make synaptic contact with postganglionic neurons located in the myenteric plexus of the intestinal wall. A viscerotopic organizational map also exists here, with the neurons innervating the small intestine being localized in the medial part of the nucleus.

Gastrointestinal peptides and satiation: introduction

Peptides secreted from the enteroendocrine cells of the small intestine signal negative-feedback information from meal-related food stimuli from the periphery to the brain. Although many gastrointestinal peptides are hypothesized to have satiation effects, for most of these, the physiological status of their satiation action is uncertain. Natural physiological endocrine effects are assessed using six criteria: (a) the plasma levels of the molecule change during meals; (b) cognate receptors for the molecule are expressed at its site of action; (c) administration of the molecule to its site of action in amounts that reproduce prandial levels at that site are sufficient to cause satiation; (d) compounds eliciting the secretion of the molecule produce effects similar to its administration; (e) the inhibitory effects on eating occur in the absence of abnormal behavioral, physiological, or subjective side effects; and (f) premeal administration of selective agonists and antagonists to the receptors for the molecule produce effects on eating consistent with their receptor pharmacologies; for example, an antagonist should delay eating if the molecule's role is to initiate eating or cause an increase in meal size if the molecule is regarded as a meal termination signal. These six criteria are considered below for CCK, GLP-1, and PYY.

CCK

CCK is released from the duodenal and jejunal I-cells in response to nutrients. In 1973, Gibbs et al. [84] reported that intraperitoneal injections of CCK-8 rapidly decreased food intake in rats. The effect seemed specific because CCK-8 did not decrease water intake or produce signs of malaise. The mode of action of CCK-8 on eating in rats was originally thought to be endocrine, a hypothesis based on other models of peptide action (e.g., pancreatic enzyme secretion). Moreover, in monkeys and humans [85], CCK plasma levels increase soon after meal onset. Subsequent research in rats disconfirmed this hypothesis because

(a) plasma CCK did not increase during meals in rats; (b) much more CCK-8 had to be infused intravenously to inhibit eating than to, for example, stimulate pancreatic exocrine secretion [86]; (c) intravenous infusion of a monoclonal antibody to CCK abolished the pancreatic response to food, but it did not change eating, whereas a small-molecule CCK receptor antagonist did increase eating [87]; and (d) the inhibitory potency of CCK-8 was markedly reduced or abolished after infusion into the portal vein or into the systemic circulation [88–90]. Thus, it was concluded that CCK-8 inhibits food intake in rats through a paracrine mode of action. More recently, it has been shown that the endogenous form of CCK is CCK-58, and it and other larger forms of CCK were much more potent than CCK-8 when administered systemically, suggesting that CCK-58 may act in part via an endocrine mode of action in rats, as well.

Local infusion experiments indicate that CCK acts in the area of the pylorus and proximal duodenum to elicit satiation in rats [91]. Selective abdominal vagal deafferentation further identifies the vagus as the route of the CCK satiation signal to the brain.

Intravenous CCK infusion was first reported to decrease eating in humans by Kissileff et al. in 1981 [92], but in view of the relatively high doses, however, it was uncertain whether the effects were physiological. Follow-up studies using clearly physiological infusion concentrations, however, indicated that that CCK decreased eating in both lean and obese subjects without illness or other adverse effects [85, 93]. Another crucial criterion for physiological function, that antagonism reverses the satiating effect of the exogenous peptide, and when administered alone, stimulates eating, was soon fulfilled in both rats and humans [94, 95].

CCK's satiating effects are mediated through interactions with CCK-1 receptors [96–100]. The Otsuka Long-Evans Tokushima fatty rat (OLETF) rat bears a spontaneous null mutation of the CCK-1 receptor gene and is hyperphagic and obese [101, 102]. Moran et al. [103] found that OLETF rats consumed larger meals and did not decrease eating after CCK injections. OLETF rats also showed a deficit in their eating responses to dietary fat in that they did not quickly compensate when switched to a high-calorie/high-fat diet, leading to an exacerbation of their hyperphagia [104] nor did they reduce intake in response to intragastric loads of fat [104]. Covasa and Ritter [105] extended these findings by showing that OLETF rats have deficits in the satiation response to a variety of intestinal nutrient infusions, and when reductions in intakes were present, CCK-1 receptor antagonists did not affect them. However, work by Bi and Moran indicates that much of the eating phenotype of OLETF rats stems not from loss of intestinal CCK signaling but from loss of neuronal CCK signaling via CCK-1 receptors in the paraventricular nucleus of the hypothalamus.

Importantly, treatment with CCK-1 receptor antagonists decreased the satiating potency of ingested food but did not abolish it. Thus, CCK must interact with other mechanisms to control eating. Synergistic interactions between CCK and several other negative-feedback signals have been identified, including orosensory nutrient stimuli [106], gastric volume [107], leptin [108], small-intestinal 5HT [109, 110], apolipoprotein AIV [111], pancreatic glucagon [112], amylin [113], and insulin [7].

CCK also changes the expression of a number of peptides and receptors in the abdominal vagal afferents, although these have not been shown yet to be part of CCK satiation. For example, CCK-8 increases the expression of cocaine and amphetamine receptor transporter (CART) [114] and decreases the expression of MCH [115] in the vagus. Finally, studies using c-Fos expression as an indicator of neuronal activity showed that in addition to the dorsal vagal complex, CCK-8 also activates neurons in the ventrolateral medulla, parabrachial nucleus, and forebrain nuclei including the central nucleus of the amygdala, paraventricular, dorsomedial, and arcuate nuclei of the hypothalamus. The roles of these presumably polysynaptic projections in the processing and integration of the peripheral information produced by peripheral CCK through the stimulation of vagal afferent fibers is still under investigation.

GLP-1

Peripheral GLP-1 is secreted from enteroendocrine L-cells located mainly in the jejunum, ileum, and colon. However, duodenal food stimuli can elicit GLP-1 secretion via neurohumoral reflexes mechanisms, which most likely lead to the increases in GLP-1 plasma levels rise within minutes of the onset of meals. The vagus is an important component of these reflexes: (a) glucose- and fat-induced GLP-1 release was greatly reduced in rats by bilateral subdiaphragmatic vagotomy [116] and (b) postprandial GLP-1 secretion was blocked by pharmacological blockade of vagal signaling with muscarinic receptor (M1 and M2) or gastrin-releasing peptide antagonists [117]. Both M1 and M2 muscarinic receptor subtypes are expressed in L-cells and are thought to mediate neural regulation of GLP-1 secretion [117].

The GLP-1 receptor is a G-protein-coupled protein and is expressed widely throughout the periphery (enteric nervous system, vagal nerves, stomach, small and large intestine, adipose tissue, kidneys, etc.) and the brain. Whether GLP-1 acts in the periphery, in the brain, or both to elicit satiation is not entirely clear. In the periphery, it probably acts at least in part in a paracrine manner in rats because intraperitoneal

GLP-1 infusion decreased eating in a situation in which intraportal GLP-1 infusion did not [118]. Furthermore, once GLP-1 is released from the L-cells, it is rapidly degraded by the enzyme dipeptidyl peptidase IV (DPP-IV), which is expressed on capillary vessels throughout the body. If GLP-1 survives the degradation by DPP-IV, it may reach the GLP-1 receptors in the brain where it may act to elicit satiation.

Additional evidence that GLP-1 acts in the periphery to elicit satiation comes from antagonist studies showing that central administration of the potent and selective GLP-1 receptor antagonist exendin (9–39) did not block decreases in eating produced after peripheral administration of GLP-1 but did block decreases in eating after central injection of GLP-1. Moreover, exendin (9–39) administered in the periphery blocked the decreases in eating caused by endogenous GLP-1 secreted in response to intestinal nutrient infusions and peripheral GLP-1 administration. The enteric nervous system or the vagal afferents could be sites where peripheral GLP-1 can act. This is supported by evidence that subdiaphragmatic vagotomy abolishes the satiating action of intraperitoneal GLP-1 infusion, although not that of GLP-1 infusion into the hepatic portal vein.

GLP-1 is also synthesized by neurons of the NTS, which then project to forebrain structures including the hypothalamus, hippocampus, and cortex, all of which express GLP-1 receptors. The relative contribution of central and peripheral GLP-1 to central GLP-1 receptor stimulation remains a matter of active research. For example, although GLP-1 administration in the lateral or fourth ventricle inhibits eating, the caudal brainstem, however, is sufficient for the inhibitory action of GLP-1 on eating because intraperitoneal or fourth ventricular injections of GLP-1 elicit satiation in decerebrate rats. Both peripheral and central injections of GLP-1 induce c-Fos expression in the PVN, and GLP-1 injections in the paraventricular nucleus, but not the arcuate, decrease eating, suggesting that peripheral and central injections of GLP-1 may decrease eating via different neuronal mechanisms. Local peripheral infusions of exendin (9–39), such as done with CCK receptor antagonist, would be very helpful in resolving this issue.

High-fat diet decreases the eating-inhibitory effect of GLP-1 by diminishing its meal-related secretion or by reducing the number of L-cells in the small intestine. Obese subjects also show increases in DPP-IV activity.

GLP-1 decreases eating by reducing meal size, rather than meal frequency. Meal size was decreased when 5-min GLP-1 infusions were administered at the onset of the first meal size in spontaneous eating rats [119]. Chelikani et al. [120], however, showed that 3-h intrajugular infusions of GLP-1 decreased both meal size and meal frequency. This discrepancy in results may be due to the fact that Chelikani et al. used a less physiological approach by administering GLP-1 by constant infusions.

PYY

PYY is secreted mainly by the same L-cells that also secrete GLP-1. As with GLP-1, the secretion is biphasic, initially stimulated by atropine-sensitive neural projections from the proximal small intestine, followed by direct nutrient stimulation. Also, like GLP-1, it is secreted mainly in response to lipids and carbohydrates. PYY (1–36), the secreted form, is inactive until proteolyzed to PYY (3–36) by DPP-IV. In the periphery, the effects of PYY (3–36) on eating arise from its interactions with inhibitory GPCR Y2R receptors.

Batterham et al. [121] initially reported that intravenous infusion of PYY (3–36) reduced eating in humans. The design was unusual, however, in that infusions were done during the inter-meal interval and plasma PYY (3–36) levels had returned to basal levels before the test meal, thus limiting its use as a satiation test. It also remains unclear whether physiological doses of PYY (3–36) are sufficient to acutely inhibit eating in rats or humans. Beglinger and colleagues [122, 123] found that only PYY (3–36) infusions that elicited plasma PYY (3–36) concentrations greater than those elicited by a large meals inhibited eating in humans. Furthermore, PYY (3–36) infusion often led to nausea, suggesting that the decreases in eating may simply represent a nonspecific aversive effect. In contrast, meal-contingent PYY (3–36) infusions reduced meal size in rats in the absence signs of aversion [124]. Furthermore, Abbott et al. [125] reported that injection of a Y2R antagonist into the arcuate nucleus reduced the effect of intraperitoneal PYY (3–36) injection and when given alone increased eating, which both further supports the hypothesis that endogenous PYY (3–36) is a normal physiological satiation signal and suggests the arcuate nucleus of the hypothalamus as its site of action. That may not, however, be the only site of action because the vagus also expresses Y2R, and vagotomy was reported to block the effect of intraperitoneal injections of PYY (3–36) [126].

Brain metabolic sensing and satiation

Introduction

As described in the introduction and Figure 2, energy homeostasis is regulated by feedback from circulating energy metabolites and hormones involved in energy

metabolism and energy storage, which act on a widely distributed set of peripheral and central neurons, dubbed as "metabolic-sensing neurons". Many of these signals, for example, blood glucose, reflect meal-related increases in absorption, energy metabolism (e.g., tissue glucose uptake, gluconeogenesis, etc.), and energy storage (blood glucose is usually increased in obesity). Others, for example, FFA and leptin, may reflect mainly energy storage. We focus on brain metabolic-sensing neurons, for which the most information is available.

Identification of metabolic sensors is a classic aspect of the study of homeostatic eating. Until recently, however, this research largely neglected how these mechanisms affect meals, relying instead on measures of total food eaten over days or longer periods. As a result, the evidence that these feedback selectively control satiation was mainly indirect, based on the overwhelming evidence that the homeostatic control of eating is affected mainly by changes in meal size, summarized in the introduction. This disconnect between homeostatic control mechanisms and behavior is reflected in our review. Happily, however, recent work has begun to not only remedy this error but also reveal the neural mechanism through which metabolic nutrient sensing and gastrointestinal nutrient sensing converge to generate an integrated satiation signal.

Which brain metabolic-sensing neurons contribute to satiation?

Introduction

Research beginning 60 years ago has identified and characterized the activity of metabolic-sensing neurons in the hypothalamic arcuate, ventromedial nucleus, and lateral hypothalamic area. More recently, metabolic-sensing neurons also have been identified in the caudal brainstem [127]. According to the theory, when extracellular levels of the sensed metabolites or hormones reach a certain level, these neurons become either excited or inhibited and set in motion neural networks that control eating, energy expenditure, and metabolism. As our review reflects, however, demonstrations that metabolic-sensing neurons play a normal phasic or tonic physiological role in the control of meal size are still lacking.

Brain glucose sensing

Jean Mayer's [128] classic glucostatic theory posited that brain glucose utilization controls eating. Since then, important milestones including identifying potassium as a key player in this process [128], electrophysiological recordings from ventromedial and lateral hypothalamic neurons that were glucose-excited (GE) or glucose-inhibited (GI) [129–131], and further characterization of the K^+-channel involved in neuron's response to glucose [132] provided a solid basis for experiments in the last two decades aimed at identifying how neurons sense glucose and their roles in health, obesity, and diabetes.

Ventromedial and arcuate nuclei glucose-sensing neurons alter their action potential frequency in response to changes in extracellular levels of glucose [133, 134]. GE neurons increase, whereas GI neurons decrease their action potential frequency in response to increases in extracellular glucose from 0.1 to 2.5 mM glucose [133]. Thus, their dynamic range spans the normal range of extracellular glucose levels in the brain, which are about ~10% of systemic blood glucose levels [135], and they are plausible normal physiological controls of eating. GE neurons require glucose metabolism for excitation [134, 136, 137]; however, GI neurons comprise distinct populations, which either require glucose metabolism [136, 137] or sense the glucose molecule directly [138].

GE neurons sense glucose by a mechanism analogous to that operating in pancreatic β-cells [134]. Glucose enters the cell via GLUT-2 transporters, is phosphorylated by glucokinase (GK), and is metabolized in the TCA cycle to increased intracellular levels of ATP. ATP inhibits ATP-inhibited K^+ channels (K_{ATP} channels), and the resultant Ca^{2+} influx depolarizes the cell [139]. The concentration-response relationship for both K_{ATP} channel currents and action potential frequency of ventromedial nucleus GE neurons are steep, linear relationships for glucose concentrations between 0.1 and 1.5 mM. The slope then decreases sharply, and the concentration-response function plateaus between 2.5 and 5 mM glucose [140], i.e., well above physiological levels. These data strongly suggest that the K_{ATP} channel on GE neurons plays an important role in physiological brain glucose sensing. Current data suggest that there may be multiple subtypes of GE neurons utilizing alternate glucose-sensing strategies, for example, sensitive or not sensitive to the α-subunit of AMPK, an important cellular fuel gauge [141, 142].

The phenotype of arcuate nucleus GE neurons remains controversial. Some studies showed that the electrical activity of proopiomelanocortin (POMC) neurons stimulated with glucose is consistent to that of GE neurons [141, 143] and correlated with changes in energy status [144]. Several observations, however, indicate that POMC neurons are not GE neurons: (1) GE neurons are not present in the region of the arcuate nucleus generally designated

as the location of POMC neurons [140]; (2) immunohistochemical evaluation following electrophysiological recordings from GE neurons did not reveal POMC-positive neurons [140]; and (3) intracarotid glucose injections did not increase levels of c-Fos expression in POMC neurons [145].

GI neurons sense glucose differently than GE neurons. Although GLUT-2 and GK are also involved [136, 137, 146], the downstream cascade clearly involves AMPK [147], whose activation leads to NO production and CAMKK activation [148], leading to the depolarization of the GI neurons in response to decreased glucose [147].

The physiological relevance of GE and GI neurons is uncertain. Experiments performed in the early 1990s showed that a 5–10% decline in blood glucose levels occurred 12–15 min preceding spontaneous meals in rats and that blocking the decline with glucose infusions delayed meal initiation, providing a causal relationship of declines in glucose and the onset of the meal [149]. A problem with this evidence is that changes in blood glucose do not necessarily reflect changes in brain glucose. When Levin and colleagues [135] measured glucose in the blood and the ventromedial hypothalamus in spontaneously eating rats, they failed to find a clear relationship between ventromedial hypothalamus glucose changes and either the onset or termination of spontaneous meals or between blood glucose and ventromedial hypothalamus glucose before meals. These elegant experiments exemplify how high the bar is to unambiguously demonstrate a normal physiological role of neuronal signaling in eating, a point that should be kept in mind in the following discussion of other metabolic-sensing mechanisms and of the neural networks controlling eating.

Finally, it is also important to note that (a) the emergency eating response to acute biochemical hypoglycemia involves ventromedial hypothalamus glucose sensing mediated by GK [135] and (b) it remains possible that glucose sensing in the portal vein or liver does contribute to the physiological control of meal size [150, 151].

Brain lipid sensing

Oomura et al. [152] suggested that FFA might have a facilitatory effect on glucose-sensitive neurons in the lateral hypothalamus. Wang et al. [153] later showed that there are oleic acid-excited and oleic acid-inhibited neurons in the arcuate nucleus whose activity depended on the extracellular glucose concentration. Le Foll et al. [154] showed that ~40% of ventromedial hypothalamic neurons were excited and increasing concentrations of oleic acid inhibited 30%, but the activity of the ventromedial hypothalamic neurons was not dependent on ambient glucose concentrations. Furthermore, inhibition of acyl-CoA synthetase, the initiating step in fatty acid metabolism, manipulations of CPT1 activity, and reactive oxygen species formation, or blockade of K_{ATP} channel activity or the fatty acid transporter CD36 reduced activity of only 20% of the excitatory and 40% of the inhibitory oleic acid-sensitive neurons, suggesting that oleic acid levels rather than utilization affected ventromedial hypothalamus neuronal activity through multiple pathways. FFA sensing by CD36, or fatty acid translocase/CD36, is especially interesting because CD36 is a membrane FFA receptor/transporter that does not require intracellular FFA metabolism to initiate signaling. In confirmation of the importance of such signaling, Moulle et al. [155] demonstrated that the eating-inhibitory effect of 10-min intracarotid, intralipid infusions was dependent on the expression of CD36 in the VMN and on acyl-CoA synthesis but not on fatty acid oxidation.

The initial tests of brain lipid sensing and eating involved intracerebroventricular injections of the fatty acid synthase inhibitors C75 and ceruleinin, which decreased eating and body weight in mice [156, 157]. The underlying lipid sensing mechanism is unclear. Some data suggest C75 reduces eating by decreasing pAMPK [158]. Obici et al. [159] extended these findings by showing that intracerebroventricular administration of oleic acid decreased eating, in part, by decreasing hypothalamic expression of neuropeptide Y (NPY) [159]. Obici and Rossetti [160] then suggested that fatty acid synthase inhibitors and the intrahypothalamic oleic acid infusions lead to an accumulation of LCFA CoAs in the hypothalamus that, via a negative-feedback mechanism, signal lipid availability and inhibit eating. Indeed, inhibition of CPT-1 in the arcuate nucleus increased levels of oleyl-CoA and was accompanied by low levels of agouti-related peptide (AgRP) and NPY mRNA levels. Interestingly, intrahypothalamic oleic acid did not decrease eating or increase NPY mRNA expression in rats fed with high-fat diet [161].

The physiological relevance of brain lipid sensing has not been directly tested. Because plasma FFA levels do not rise during meals, they are unlikely to serve as phasic satiation signals related to ongoing meals. FFA levels do rise in obesity [162], suggesting a possible role as a tonic weight-regulatory satiation signal. If so, however, the brain must combine this information with other metabolic sensing because FFA levels also rise during fasting.

Another issue with brain lipid utilization sensing is that the brain fatty acid oxidation occurs in astrocytes, not neurons [163–165]. It is therefore possible that brain lipid

sensing is based on neuronal uptake of ketone bodies produced in astrocytes. Le Foll et al. [24], using electrophysiology and microdialysis techniques, confirmed this hypothesis by showing that (a) plasma and ventromedial hypothalamic plasma β-hydroxybutyrate levels were higher in rats during the first hour of high-fat diet feeding following an overnight fast; (b) as ketones can have an overriding, excitatory action on either glucose or oleic acid stimulation of ventromedial hypothalamic neurons, the rats decreased eating; and (c) this eating-inhibitory effect was reversed by inhibition of ketone production by 3-hydroxy-3-methylglutaryl-CoA synthase inhibitor.

Brain amino acid sensing

Initial studies by Cota et al. [166, 167] suggested an important role for hypothalamic leucine sensing and downstream changes in mTOR and S6K1 in the control of eating and body weight as well as during diet-induced obese states. Work by Blouet and colleagues [127, 168, 169] has begun to unravel the eating effects of leucine sensing in ventromedial and arcuate nuclei of the hypothalamus and NTS in the control of eating. They demonstrated that in the fasted rats, (a) cerebrospinal fluid leucine concentrations were 26 times lower than plasma leucine levels; (b) ingestion of a high-leucine meals raised the CSF leucine levels; (c) intraventricular infusions of leucine in the third or fourth ventricle decreased eating; (d) third ventricle injection of inhibitor of leucine metabolism (i.e., ketogenesis) decreased eating. These data strongly support the hypothesis that brain leucine sensing can physiologically control eating, at least under certain dietary conditions. An interesting point was that third ventricle leucine injections did not affect meal size after the first meal, but led to a large decrease in meal frequency, whereas fourth ventricle leucine injections decreased meal size and not meal frequency. This suggests that NTS leucine sensing may be more important for the tonic contribution of satiation related to energy homeostasis than ventromedial/arcuate nuclei leucine sensing.

Brain insulin sensing

The hypothesis that insulin might provide a tonic signal linking adipose tissue energy stores to the control of eating arose from (a) Bagdade et al.'s [170] reports that both basal, fasting insulin levels and glucose-stimulated insulin levels are linearly correlated to adipose tissue mass, which they estimated by percent ideal body weight, and (b) a similar correlation between body weight and basal insulin in rats [171]. Insulin, as with leptin, has been demonstrated to regulate eating. Plasma insulin is low during fasting and increases mainly during and after meals. Both basal and prandial insulin levels are direct functions of the amount of white adipose tissue in the body [170, 172, 173]. Soon after, Woods and colleagues [174–176] reported that insulin infusions into the third ventricle or near or directly within the mediobasal hypothalamus decreased eating and body weight in baboons and rats. The responses were dose-dependent [174, 175] and apparently not related to illness [177]. Further important discoveries included the following: (a) insulin entered the brain; (b) brain insulin levels in the cerebrospinal fluid were higher in obese rats than lean rats; (c) insulin receptors exist in the brain; and (d) insulin receptors exist on NPY/AgRP and POMC neurons in the arcuate nucleus as well as other brain areas implicated in the control of eating (for a review, see [178]). Despite this wealth of data, however, the hypothesis that endocrine insulin functions, as a normal physiological endocrine adiposity signal, lacks crucial support: (a) whether physiological intravenous or central doses of insulin are sufficient to inhibit eating either acutely or tonically is not clear [179]; (b) although there are reports that acute central insulin antibody treatment increase eating [180], there are no demonstrations that chronic central insulin increases eating (there is a report that brain-specific transgenic deletion of insulin receptors increases eating and body weight in female mice, but this appeared not to be the case in males [181]; (c) basal levels of plasma insulin did not change in association with weight-regulatory eating responses in rats recovering from forced overweight [182] and in other situations [183].

Brain leptin sensing

Leptin is an adipokine hormone secreted by white adipose tissue. Leptin and leptin receptors were discovered in the search for the hormonal mechanism accounting for the hyperphagia/obesity phenotypes of the *ob*/*ob* (leptin-deficient, now known as Lepob) or *db*/*db* (leptin receptor-deficient, Leprdb) mice [184, 185]. Circulating leptin is taken up into the brain [186], and leptin receptors are highly expressed in NPY/AgRP and POMC neurons in the arcuate nucleus as well as in the dorsal vagal complex and other areas [187–189]. NTS neurons express leptin receptors, and fourth ventricular and NTS injections of leptin inhibit eating, although whether this is a meal size effect has not been tested [190].

Basal leptin levels are linearly correlated to fat mass. Although plasma leptin levels display a marked circadian variation, they are not affected by individual meals, so that their relation with fat mass is maintained consistently [191]. Evidence suggesting that leptin is a tonic satiation signal includes (a) three groups of investigators reported that doses of peripheral and central leptin that inhibited eating did so by reducing the size of the rats' spontaneous meals without affecting the number of meals consumed [192] (fourth ventricular and NTS injections of leptin inhibit eating [190], but whether this is a meal size effect has not been tested); (b) chronic peripheral leptin treatment also reduced eating by selectively decreasing meal size [13, 14]; and (c) the hyperphagia due to genetic impairments of leptin signaling in rats and mice were characterized by meal size-specific alterations. Thus, the hyperphagia of *ob*/*ob* mice and Zucker rats (with deficits in leptin receptors) are both characterized by chronic increases in the size of spontaneous meals [12, 193]. There is mixed support for the hypothesis that these effects reflect a normal physiological function: (a) physiological doses of leptin appeared sufficient to inhibit eating in mice [179]; (b) in obese humans tested after 10% weight loss, infusing leptin in amounts sufficient to reproduce pre-weight loss levels reversed subjective measures of meal-induced satiation [92]; (c) infusion of a leptin receptor antagonist over 1 week increased eating and weight gain [194]; (d) moreover, when infused together with leptin, the antagonist prevented the anorectic and weight-reducing responses to leptin as well as the increase in leptin signaling gain [194]. Because the antagonist neither stimulated leptin-induced STAT3 phosphorylation (see below) nor reduced the basal levels of phosphorylated STAT3, Zhang et al. concluded that in both tests, the agent actively antagonized the rat leptin receptor. Whether plasma leptin levels encode adiposity information in all situations remains unclear, however, as several dissociations among adiposity, leptin levels, and weight-regulatory eating have been reported [182, 183].

Leptin is a pleiotropic hormone that activates different intracellular signaling cascades in the brain. When it binds to the long signaling form of its receptor ($Lepr^b$), it recruits the Janus kinase (JAK). JAK binds to and phosphorylates $Lepr^b$ [195], which activates STAT3. Once phosphorylated, STAT3 binds to *pomc* and *agrp* promoters, stimulating POMC and inhibiting AgRP expression [196, 197]. Leptin via $Lepr^b$ also activates PI3K, which induces the synthesis of PIP3 from PIP2 [144, 198, 199]. Accumulation of PIP3 leads to PDK1 activation, which further activates protein kinase B (PKB, also know as AKT).

Interestingly, the leptin- and insulin-signaling pathways converge in PI3K activation [189, 200]. AKT, one of the PDK1 downstream targets, has an important role in the regulation and activation of many proteins and transcription factors, such as FoxO1 [201, 202], AMPK [141], and mTOR [166]. FoxO1 acts as an inhibitor of POMC expression [203]. Leptin and insulin also regulate the AMPK signaling pathway in the hypothalamus [141]. Proteins involved in this pathway are hypothesized to sense energy status and are activated by energy deficiency [204]. Leptin and insulin inhibit the activation of AMPK and its downstream targets.

The mTOR pathway is also thought to be involved in the hypothalamic regulation of eating [166]. Intact mTOR signaling is necessary for the eating-inhibitory effect of leptin [166]. In contrast to AMPK, hypothalamic mTOR expression is higher in situations of energy surplus, perhaps mediated by both leptin [166] and insulin signaling [205].

How does the brain integrate satiation feedback?

Introduction

The neurochemical and neurophysiological characterization of the neural pathways underlying the effects of the hormone leptin on eating led to important insights into the neural networks controlling eating [206, 207]. In particular, both in the hypothalamus and in the DVC in the caudal brainstem, neurons that responded to leptin were also activated by other metabolic and gastrointestinal sensors. These findings established that the regulation of eating, energy expenditure, and adipose tissue metabolism is mediated by a distributed integratory neural network involving bidirectional communications between the forebrain and the hindbrain rather than by the hypothalamus alone. In this section, we first review some of the key neurochemical signals involved in eating. An unfortunate aspect of this work is that it often does not link the functions described to meal control. Therefore, we close by describing progress in understanding the convergence of forebrain neural networks on caudal brainstem control of networks mediating satiation.

Perhaps the most investigated neurons with respect to regulation of eating are the POMC and NPY/AgRP neurons in the arcuate nucleus. POMC neurons project throughout the brain, where they release α-melanocyte stimulating hormone (α-MSH), which binds to melanocortin receptors 3 and 4 (MCR3 and MCR4). NPY/AgRP neurons release AgRP, an α-MSH inverse agonist that acts at the MCR to

counteract the effects of α-MSH. NPY exerts its effects on eating via NPY receptors (NPYR) (1, 2, 4, and 5), which are independent of MCR. The discovery of this ARC microcircuit greatly accelerated the progress in understanding the neural control of eating, some of which we review below.

POMC neurons

Mouse models of *Pomc* and *Mc3r/Mc4r* deficiency display hyperphagia and obesity [208–210]. Central administration of melanocortin receptor agonists dose-dependently decreases short-term eating [211]. Both genetic and pharmacological studies suggest that long-term activation of the melanocortin system may chronically decrease eating and body weight. Indeed, transgenic mice overexpressing melanocortins show decreased eating and lower body weight gain and adiposity than controls [212, 213]. Long-term effects on body weight have also been investigated using POMC-adeno-associated virus (POMC-AAV)-mediated overexpression at specific sites within the brain. For example, hypothalamic POMC-AAV expression in the arcuate nucleus, but not at other hypothalamic sites, was sufficient to reduce the hyperphagia in leptin-deficient, obese rats [214]. POMC-AAV injections in the NTS and VTA also had the same inhibitory effect on eating and body weight gain in diet-induced obese rats [215, 216]. Aponte and colleagues [217] showed that optogenetic stimulation of POMC neurons in the arcuate nucleus was required for a long-term (24 h) rather than a short-term (2 h) to decrease eating. Although this does not agree with previous findings about the latency of POMC neuronal activation to reduce eating, some alternative explanations should be considered: (a) POMC peptides diffuse slowly to their targets in the brain; (b) activation of POMC neurons decrease eating only under certain conditions (e.g., the onset of the dark cycle); (c) specific stimulation patterns may be required for POMC neurons to have effects on eating; and finally, (d) injections of a lentiviral gene delivery vector carrying a gene construct for production of α-MSH into the arcuate nucleus of the hypothalamus in mice led to a sustained attenuation of diet-induced obesity [218].

The NTS also contains a small but functionally important number of POMC neurons [219, 220]. To our knowledge, there is only pharmacological evidence that establishes the sufficiency of arcuate nucleus vs. NTS POMC neurons in the regulation of short-term eating. That is, Seeley et al. [221] demonstrated that injections of the nonselective MC4R antagonist SHU9119 into the third ventricle of rats increased 4- and 24-h food intake. However, they did not test the effects of antagonist administration in conditions in which the third ventricle aqueduct was blocked, which would eliminate the possibility that the effect on eating was due to blockade of MC4R in the NTS. The necessity of arcuate nucleus POMC neurons in the regulation of short-term eating is still unclear.

In contrast to the case of the arcuate nucleus, more evidence supports a necessary role of the NTS POMC neurons in the regulation of eating behavior. Injections of the MC3/4R agonist MTII into the caudal brainstem are sufficient to decrease eating [222–224]. Furthermore, the effects on spontaneous eating following administration of MTII near the caudal brainstem or into the DVC are due to changes in meal size [225]. Using DREADD pharmacogenetic technology, POMC neurons were activated in either the arcuate nucleus or the NTS. Chronic, but not acute, activation of arcuate POMC neurons reduced eating, and their ablation resulted in hyperphagia and obesity. In contrast, acute stimulation of NTS POMC neurons rapidly reduced eating, and ablating this POMC neuronal population did not produce any obvious changes in eating behavior or body weight [226]. These results are consistent with the view that the POMC neurons in the arcuate nucleus are critical for maintaining long-term energy homeostasis and that the NTS POMC neurons contribute to short-term feeding control, i.e., probably in response to gastrointestinal satiation signals.

An interesting aspect in the identification of the segregation of roles between arcuate and NTS POMC neurons was revealed by a study performed by Zheng et al. [227], in which an adenoviral transfer vector expressing EGFP only when transduced into cre-expressing cells was used to exclusively label POMC neurons. They showed that unilateral injection of this tracer resulted in GFP-positive axon profiles in the NTS and other areas of the caudal brainstem. To determine the proportion of NTS terminating α-MSH fibers that arises from arcuate-POMC neurons vs. NTS-POMC neurons, α-MSH immunoreactivity in the NTS and vicinity of intact rats were compared with those of decerebrate rats. In intact rats, very fine axon profiles and a moderate number of relatively large caliber axon profiles were present throughout the NTS. By contrast, in decerebrate rats, most of the larger-caliber fibers were missing and only the fine immunoreactive fibers remained in this region. These results suggest that NTS neurons are supplied with endogenous α-MSH both by arcuate- and NTS-POMC neurons.

POMC neurons project to multiple brain sites, both intrahypothalamic and extrahypothalamic. The network of the POMC circuit can be divided into leptin-, insulin-, or 5HT-sensitive neurons [228, 229], and to a lesser extent,

leptin- and insulin-responsive neurons [230]. To release bioactive signaling molecules, POMC neurons integrate afferent signals until a depolarization induces the release of peptidergic vesicles and granules in a calcium-dependent manner [231, 232]. According to classically described mechanisms, boutons in presynaptic terminals should release neuropeptides onto other neurons expressing the appropriate receptors. Interestingly, peptides released from POMC neurons can be detected at sites devoid of postsynaptic receptors and at nonsynaptic sites as well [233–236]. Accordingly, POMC neurons may participate both in synaptic and volume transmission in the brain [237, 238].

Given that POMC neuronal fibers are present around the ventricles through the entire rostrocaudal extent of the brain, an important aspect of these phenomena is that the neuropeptides released by POMC neurons can enter the cerebral ventricles and act at sites lacking direct POMC synaptic control [239–241]. This possibility was confirmed by findings that ICV administration MTII can reduce or increase eating, respectively [242–244]. MTII reduced intake of scheduled glucose meals and of overnight spontaneous pellet intake [245]. These extended previous findings that MTII decreases eating during the dark phase [222, 223]. MTII selectively reduced meal size and meal duration, with no change in meal frequency [245], suggesting that MTII reduces eating by increasing the satiating potency of negative-feedback signals critical to satiation. Interestingly, however, MTII did not affect the reductions in eating produced by intraduodenal glucose infusions [245], suggesting that MTII does not modulate the potency of gastrointestinal satiation signals. Thus, more work is required to determine the functional significance of α-MSH signaling.

Central melanocortins have been proposed as downstream mediators of the effects of leptin [246]. The first evidence for this was a report of Seeley et al. [221] that administration of the MC4R antagonist SHU9119 into the third ventricle immediately before a leptin injection completely blocked leptin's effect on eating. This appeared selective to leptin because SHU9119 did not block the effect of third ventricle administration of GLP-1 [221]. The interaction appears to occur within hypothalamic POMC neurons because they express leptin receptors [187] and leptin activates them, as shown both by increases in c-Fos expression [247] and by electrophysiological recordings [188]. Leptin administration in nondeprived rats increased POMC mRNA in the hypothalamus [248]. Mice selectively deficient for leptin receptors on POMC neurons were less obese than mice globally deficient in leptin receptors [249], and the difference developed as a result of decreased energy expenditure, rather than increases in eating behavior. Thus, MC4R signaling is selectively involved in leptin's effect on eating.

There are several indications that caudal brainstem MC4R contribute to the mediation of eating by hindbrain-delivered leptin. Caudal brainstem application of leptin decreased eating similarly to effects of fourth ventricular or NTS injections of MTII [190, 250, 251], and this was reversed by MC4R antagonist pretreatment [250]. These data suggest that caudal brainstems MC4R are part of the downstream mediation of hindbrain-induced effects on eating. POMC-EGFP mice were used to investigate whether the NTS POMC neurons are also leptin-signaling neurons. The advantage of this method is that the POMC neurons can be unambiguously identified in the NTS, whereas immunocytochemical or in situ hybridization methods failed before. Systemic leptin induced pSTAT3 immunoreactivity in NTS POMC-EGFP neurons that were located mostly caudal to the AP [252]. These results indicate that NTS POMC neurons are leptin-signaling neurons.

The eating-inhibitory effect of insulin may also involve POMC neurons. Injections of SHU9119 in the third ventricle attenuated the reduction in eating caused by insulin injections to the third ventricle [253, 254], and after a prolonged fast, POMC expression in the arcuate nucleus was attenuated by insulin, consistent with previous findings that low levels of insulin in diabetic rats was accompanied by low levels of POMC expression, and it is restored by systemic insulin treatment [254]. Insulin receptors are expressed on POMC neurons in the arcuate nucleus [253], although whether they are also expressed by POMC neurons in the NTS or other regions of the hindbrain has not been investigated. In the arcuate nucleus, insulin hyperpolarizes POMC neurons [141, 189, 228, 255]. In contrast to the effect of leptin receptor lesions, the selective ablation of insulin receptors in POMC neurons had no effect on eating [189]. In another study, however, when insulin receptors were re-expressed in insulin receptor KO mice, food intake increased [256].

Numerous lines of evidence suggest that 5HT-2CR in POMC neurons constitute a key node governing eating: (a) POMC neurons in the ARC express 5HT-2CR and receive input from other 5HT-immunoreactive terminals [257, 258]; (b) 5HT-2CR agonists stimulated POMC neurons in the arcuate nucleus [259, 260]; and (c) 5HT-2CR reactivation specifically in the POMC neurons of otherwise 5HT-2CR-null mice improved energy homeostasis [261, 262].

Serotonin's effects on eating may involve several mechanisms: (a) direct activation of arcuate POMC neurons via 5HT-2CR; (b) indirect 5HT-1BR-mediated disinhibition of POMC neurons via blockade of local inhibitory

inputs; and (c) direct inhibition of AgRP neurons [229, 257, 263]. Recent data demonstrate that 5HT-2CR signaling in the POMC neurons is required to maintain energy homeostasis. Selective loss of 5HT-2CR signaling in POMC neurons resulted in hyperphagia and sensitization to diet-induced obesity and blunted the effects of D-fenfluramine and mCPP to suppress eating [264]. These data support the role of POMC neurons in the arcuate nucleus in the control of eating, in contrast to the demonstration that leptin receptor signaling via POMC neurons did not affect eating, only energy expenditure [255, 265]. It is important to note that 5HT-2CR and leptin receptors are expressed by anatomically and functionally distinct POMC neurons in the arcuate nucleus, but these populations converge on similar second-order neurons, including those expressing MC4R [228, 229].

Finally, using an in vitro culture system of neurons expressing fluorophores under control of *pomc* transgenes that allowed electrophysiological characterization of identified neurons, Hentges et al. [266, 267] showed that there are many GABAergic and few glutamatergic subpopulations of POMC neurons. Consistent with GABA release, approximately 40% of POMC neurons express GABA-synthesizing enzymes GAD65 and GAD67 [266, 268]. Immunohistochemically, POMC axon terminals were readily identified as GABAergic or glutamatergic. To our knowledge, most of the evidence supporting a role of GABA receptors on POMC neurons on eating comes from one study in which mice that lacked metabotropic GABA receptors on POMC neurons were found to be heavier than WT mice, but only when the mice were placed on high-fat diet, and interestingly, it was more pronounced in male mice than in female mice [269].

AgRP/NPY neurons

The role of AgRP in the regulation of eating has been examined in a number of different rodent models. Some of the main findings are the following: (a) AgRP expression in the arcuate nucleus is increased during fasting and in mice with genetic leptin deficiency [248, 270, 271]; (b) both overexpression of AgRP and intracerebroventricular AgRP administration increased eating [272, 273]; (c) selective acute activation of AgRP neurons using DREADD pharmacogenetic technology [274] or optogenetic technology [217] induced voracious eating; (d) selective pharmacogenetic antagonism of AgRP neurons reduced eating [274]; (e) interestingly, however, reduction of hypothalamic AgRP expression by RNA interference decreased body weight independent of changes in eating [275].

As with POMC neurons, leptin and insulin modulate the activity of AgRP neurons. Deletion of leptin receptors on AgRP neurons increases body weight and adiposity [276]. When leptin receptors were deleted from both POMC and AgRP neurons, the mice became more obese, leading the authors to conclude that POMC and AgRP neurons work synergistically in response to leptin [276]. Insulin also acts on AgRP neurons in the arcuate nucleus [141, 189]. Unlike leptin, however, selective ablation of insulin receptors on AgRP neurons had no effect on body weight or food intake [189]. Several groups devised strategies to ablate AgRP neurons to determine the importance of these neurons in eating and body weight regulation [277–280]. Consistent with the data reviewed above, the results indicate that AgRP neurons are important for initiating meals and maintaining eating during meals in adult mice. Interestingly, however, adaptive mechanisms can compensate for the germ line AgRP lesions.

Two hypotheses have been proposed to account for AgRP's role in eating. First, AgRP exerts its action by blocking the binding of α-MSH to its receptor [271, 281–283]. GABA release from AgRP neurons has a direct effect on POMC cells and probably on most postsynaptic MC4R-bearing cells [188, 284, 285]. GABA co-localizes with NPY-immuno-positive axon terminals that innervate local POMC neurons in both the arcuate and the paraventricular nuclei. Electrophysiological recordings indicate that leptin inhibits release of GABA from NPY terminals that synapse on POMC neurons [188]. Based on these observations, GABA output from AgRP neurons may be important in maintaining a dynamic balance with excitatory signaling in certain postsynaptic target areas. Second, several lines of evidence suggest a melanocortin-independent pathway for AgRP. For example, AgRP still stimulated eating in MC4R-KO mice [30]. In addition, chronic blockade of the melanocortin pathway in $A^{(y)/a}$ genetic background (in which agouti protein is ectopically expressed in the brain, thereby chronically blocking MC4R signaling) failed to ameliorate the severe anorexia after ablation of AgRP neurons [285].

The role of STAT3 in AgRP neurons has been examined in an attempt to identify the signaling mechanisms behind the role of AgRP neurons in the regulation of eating and body weight [196, 203, 286]. Data indicate that (a) STAT3 activation by leptin inhibited AgRP mRNA expression levels; (b) STAT3 deletion led to mild in body-weight gain in mice, but unlike the deletions of STAT3 in POMC neurons, the effects were because of changes in energy expenditure; (c) overexpression of STAT3 in AgRP neurons increased locomotor activity, with no changes in eating [196]. As mentioned in Brain leptin sensing, the PI3K-PKD1-FoxO1

signaling pathway acts to integrate leptin and insulin signals. PI3K is required for the actions of leptin and insulin on AgRP neurons [198, 287, 288]. Deletion of PDK1 in AgRP neurons resulted in body weight loss as a consequence of decreased eating and enhanced energy expenditure [288]. Interestingly, in these same mice, leptin had an enhanced effect on eating. The specific deletion of FoxO1 in AgRP neurons was associated with decreased eating, without a change in body weight [201].

Although NPY and AgRP are secreted from the same arcuate neurons, unlike AgRP, NPY is also expressed in many other brain areas [289]. When administered into the cerebroventricular system or directly into the hypothalamus, exogenous NPY dose-dependently increases eating [290], and when it is administered chronically, body weight and body fat increase as well [290]. Whether injected into the hypothalamus [291–293] or the hindbrain [294–296], NPY elicits very-short-latency eating responses, typically seconds to minutes, and also prolongs eating once it has begun, with consequently larger meals being consumed. Thus, NPY both facilitates the onset of meals and delays satiation. Finally, the orexigenic response to NPY, similar with AgRP, is long lasting, often persisting for several hours or more [297–299].

There are data indicating that endogenous NPY also has an eating-stimulatory effect: (a) When antibodies to NPY were injected intraventricularly or directly into the VMH or PVN, food intake was decreased [300–302]; (b) administration of NPY antisense oligonucleotides or AAV to knock down endogenous NPY also inhibited eating [303, 304]. Further support for a role of endogenous NPY in eating comes from reports that fasting upregulated NPY mRNA in the ARC and NPY protein in the arcuate nucleus and paraventricular nucleus [305–308] and that these responses were reversed by refeeding [306, 309].

The dorsomedial hypothalamus is another site of NPY action. Bi [310] tested the effects of AAV-mediated NPY overexpression in the dorsomedial nucleus. Rats increased food intake by increasing nocturnal meal size and increased body weight, and being fed a high-fat diet augmented these effects. Bi also showed that in OLETF rats, which lack CCK-1 receptors and have elevated *npy* expression in the dorsomedial nucleus, NPY knockdown completely normalized nocturnal meal size [296].

The neural circuits underlying the actions of arcuate and dorsomedial hypothalamus NPY seem to differ. Arcuate NPY serves as a downstream mediator of leptin's action on eating [311, 312], whereas dorsomedial NPY signaling is independent of leptin [313] but is affected by central (i.e., neurocrine, not endocrine) CCK signaling [314]. Hypothalamic NPY, however, may also be involved in mediating endocrine CCK satiation because NPY administration in the third ventricle reduced the ability of intraperitoneal CCK to inhibit eating. This behavioral response was supported by the fact that NPY reduced c-Fos expression elicited by peripheral CCK, whereas NPY alone did not elicit any c-Fos expression by itself [312, 315]. Because dorsomedial NPY neurons project to the NTS to modulate eating [296], these data suggest that both the dorsomedial hypothalamus and NTS are sites of action for the eating-stimulatory effects of NPY.

Serotonin

Increasing (by administering a 5HT precursor) or decreasing (by lesioning) 5HT function has profound effects on eating [316]. Blundell [317] was the first to propose that the serotonergic system is involved in satiation. Indeed, 5HT function is now associated with both within-meal satiation and post-meal satiety [318]. Of the 14 subtypes of 5HT receptors now recognized, 5HT-2C and 5HT-1B receptors (2CR and 1BR, respectively) seem to mediate most of the effects of serotonergic drugs on eating behavior, apparently via increased α-MSH release and reduced release [257, 263, 319].

Transgenic mice that lack 5HT-1BR exhibit increased body weight, which seemed to represent growth rather than obesity, and increased food intake that was in proportion to weight [320]. In another study, however, 5HT-1BR-KO mice displayed increased exploratory behavior but no change in food intake [321]. Serotonin 1BR agonists produce hypophagia, which is attenuated by 1BR antagonist treatment [322, 323]. Direct infusions of a 5HT-1BR agonist into the parabrachial nucleus of the pons, a 5HT target site, potently and selectively reduced eating [324]. The behavioral sequence of satiety was also preserved, with the onset of resting being advanced [322].

The role of serotonin in the control of appetite was initially examined with the serotonin releaser and reuptake inhibitor fenfluramine and its selective enantiomer D-fenfluramine. Both drugs produced changes in eating behavior that were consistent with satiation, rather than hyperactivity and malaise [325–327]. The use of antagonists revealed that the effects of D-fenfluramine were mediated in part by 2CR receptors [328–330]. Selective serotonin 2CR agonists also increased satiation [331, 332]. Finally, mice lacking functional serotonin 2CR displayed marked hyperphagia, leading to the development of obesity [333, 334] and a normal behavioral sequence of satiety [329].

Pharmacological evidence indicates that peripheral CCK elicits satiation via brain serotonergic pathways

involving serotonin 2CR [335–337]. The pharmacological tools used, however, were not highly selective to 2CR and were not administered locally, which is important because several populations of serotonin receptors in the brain and in the periphery have been implicated in CCK satiation. Studies in mice deficient in 2CR, however, showed that these serotonin receptors are crucial in the mediation of CCK satiation [338]. Furthermore, intraperitoneal injections of doses of GLP-1 that reduced eating in wild-type mice by 50–60% had no reliable effect in 2CR-KO mice [338]. These are the first data that we know implicating serotonin in GLP-1 satiation.

Several neuropeptides and neurotransmitters are involved in serotonin's effect on eating. mCPP, a 2CR/1BR agonist, activated POMC- and inhibited AgRP-expressing neurons in the ARC [257, 260]. The downstream modulation of the melanocortin system appears to be essential to the serotoninergic regulation of eating because $A^{(y)/a}$ mice, mice treated with SHU9119, and *Mc4r*-KO mice were all insensitive to D-fenfluramine and other serotonin receptor agonists that induced hypophagia [260, 263]. Selective expression of 2CR only on POMC neurons is sufficient to normalize the hyperphagia and obesity and to attenuate responses to anorectic serotonergic drugs in 5HT-2CR-KO mice [261]. These data indicate that serotonin acting at the 2CR expressed on POMC neurons mediate much of serotonin's effects on eating and body weight.

NPY neurons receive serotonergic inputs [263, 339] and are hyperpolarized by 1BR agonists [263]. Levels of NPY mRNA and protein were decreased by pharmacological serotonin increase [340, 341]. Moreover, feeding induced by NPY administration was attenuated by D-fenfluramine [342, 343]. The inhibition of NPY/AgRP neurons by 1BR action, coupled with the activation of opposing POMC neurons by 2CR action, suggests that these receptors complement each other's effects on at least one convergent downstream pathway.

Oxytocin

In 1989, Arletti et al. [344] first demonstrated that intracerebroventricular oxytocin reduced eating and intracerebroventricular administration of an oxytocin receptor antagonist increased eating in rats. Since then, many studies have linked changes in brain oxytocin signaling with changes in eating behavior: (1) hypothalamic oxytocin mRNA expression is reduced with fasting and is restored upon refeeding; (2) eating activated oxytocin neurons in rats; and (3) reduction of oxytocin mRNA and protein in the paraventricular nucleus led mice to develop obesity, which can be rescued by oxytocin treatment [345, 346].

The mechanisms underlying the effects of oxytocin on eating are not well understood. Studies in rodents indicate that oxytocin decreases eating primarily by decreasing meal size and that hindbrain signaling is involved. Administration of an oxytocin antagonist alone stimulates eating [169, 344, 347–349] by increasing meal size [350]. Functional and anatomical data provide evidence that an interaction between oxytocin and hindbrain signaling mediates meal size.

Mice lacking oxytocin or oxytocin receptors develop late-onset obesity with little changes in daily food intake [351–353], and pair-feeding studies confirm that reductions in eating only partially account for oxytocin's effect on body weight, suggesting that oxytocin also controls energy expenditure [354, 355].

Noradrenergic neurons

Neurons in the caudal brainstem noradrenergic A2 cell group are reciprocally connected with several brain regions. Direct projections from the cortex, limbic forebrain, and hypothalamus to the A2 cell group modulate a variety of visceral responses [356]. In turn, ascending projections from the A2 neurons provide a route through which feedback from the viscera affects not only hypothalamic function but also emotional and cognitive processing [356, 357].

Different subpopulations of A2 neurons are recruited by signals that increase or decrease eating, perhaps because different subpopulations target different brain regions and/or because different combinations of adrenergic receptors are expressed in those regions [358]. A2 neurons seem to be activated in every experimental situation in which food intake is inhibited, including normal satiety [359–364]. A2 neurons are recruited in a graded manner in rats after eating, such that larger meals activate larger numbers of A2 neurons [365]. A2 neurons are also necessary for the satiating effect of CCK [351, 366, 367].

Forebrain-hindbrain neural networks for satiation

Satiation in the decerebrate rat

The general plan of a neural system for the control of the basic, rhythmic movements of eating consists of two categories of neurons: first, a network of neurons that generate

the rhythmic motor output, referred to as a central pattern generator (CPG), and second, all of the inputs to the CPG that turn it on and off. The CPG for licking, masticating, swallowing, and other eating movements are in the hindbrain. These are normally controlled by local afferent stimuli that project to the hindbrain from the mouth, gastrointestinal tract, etc., and by descending inputs from the forebrain stimuli carrying information about the current environment, metabolic state, the effects of prior ingestive experience, mood, ongoing brain activities not directly related to eating, etc. Disconnecting the caudal brainstem from the forebrain thus should reveal the capacity of afferent stimuli that project to the caudal brainstem below the level of the disconnection to control the CPG in the absence of forebrain afferent stimuli. Grill and Norgren [368] have accomplished this analysis by investigating the control of liquid intake in the chronic decerebrate rat.

The decerebrate rat, even when food deprived, does not initiate eating despite the presence of food in its environment. If liquid food is infused into the mouth through a chronically implanted oral catheter, however, the decerebrate rat initiates eating. It continues for a time and then stops, allowing the infused liquid to drain out of its mouth; importantly, it does not emit mouth movements indicative of aversion, such as when bitter food is infused. Furthermore, after eating, it grooms and then is quiescent. Thus, like an intact rat, the decerebrate rat eats meals. In addition, the size of these meals is sensitive to feedback control; for example, decerebrate rats eat more of sweeter food and eat less after CCK injection. With regard to metabolic sensing, the picture is more complicated – decerebrate rats eat more in response to 2-deoxy-D-glucose, which blocks glucose metabolism but not in response to mercaptoacetate, which blocks fatty acid oxidation. They also fail to respond to food deprivation [369]. These results demonstrate that (a) the disconnected caudal brainstem has sufficient capacity to integrate a variety of positive and negative feedback and to appropriately control the CPG for eating so as to produce a near-normal meal and (b) a variety of controls of eating and satiation depend critically on the forebrain. The next sections consider, first, some evidence concerning neural basis of the integrative capacity of the caudal brainstem and, second, how forebrain controls reach the caudal brainstem, research that represent the beginning of the construction of a complete neural network for satiation.

Caudal brainstem integrative networks

DVC POMC neurons are involved in the control of eating by CCK. In a series of experiments, Fan and colleagues [370] showed that CCK activates NTS POMC-EGFP neurons and that CCK treatment fails to elicit satiation in MC4R-KO mice. Berthoud [371] found that hyperphagia induced by the MC4R antagonist SHU-9119 injected into the fourth ventricle was characterized by increases in meal size, with no effect on meal frequency (see also [372]). The same results were reported following fourth ventricular injections of MTII. A suggested mechanism for the interaction of the melanocortin and CCK signaling systems comes from studies revealing that MC4R are expressed presynaptically on vagal afferents and postsynaptically on NTS neurons themselves [373]. Because the neurons stimulated with MTII responded with an increase in EPSP frequency, the authors concluded that MC4R signaling involves presynaptic enhancement of glutamate synaptic transmission and suggested that it is this mechanism, rather than postsynaptic activation of NTS neurons, that may account for the melanocortinergically induced decreases in eating via enhancement of vagal afferent satiation signals from the gastrointestinal tract.

Brainstem leptin signaling seems act by modulating CCK satiation. This is because Hayes et al. [374], using an RNA interference method, found that selective knockdown of *Leprb* in the NTS increased food intake and body weight and decreased CCK satiation. There is also a very similar interaction between leptin and GLP-1 satiation signaling in the NTS [375].

Finally, injections of D-fenfluramine and serotonin receptor agonists to the fourth ventricle reduced eating in decerebrate rats, suggesting that serotonin action in the caudal brainstem is sufficient to provide some level of control over food intake [376, 377].

Descending projections for satiation

Several lines of research indicate that forebrain eating-control signals do not project directly to caudal brainstem CPG but rather to circuits that integrate gastrointestinal satiation mechanisms. Perhaps the first evidence for this were reports that insulin and leptin increased the satiating potency of peripheral CCK in a dose-dependent manner [378]. These latter investigators then localized the leptin/CCK interaction at least in part to the NTS by demonstrating that leptin also increased the potencies of both CCK [379] and intragastric nutrient loads [379] to activate neurons in the NTS, as indicated by c-Fos immunochemistry.

Morton et al. [380] elegantly extended these earlier findings using Koletsky rats, which bear a global *Leprb* defect. They demonstrated that (a) these rats have

increased meal size, a decreased satiation response to exogenous CCK, and reduced c-Fos expression in the NTS after CCK injection and (b) transgenic replacement of *Leprb* specifically in the arcuate nucleus normalized all three defects. The same investigators [372] went on to identify the forebrain-brainstem projection that is likely to be involved in this interaction by showing that (a) the injection of an MC4R antagonist into the paraventricular nucleus reduced CCK satiation and (b) PVN neurons that were labeled after injection of a retrograde neuronal tracer into the NTS and collected by laser-capture microdissection express MC4R.

Oxytocinergic projections from the hypothalamus to the caudal brainstem are also involved in the control of eating. Oxytocin projections comprise ~6% of all paraventricular nucleus projections to the DVC and provide its sole source of oxytocin [361, 381, 382]. Release of oxytocin from descending paraventricular nucleus-NTS projections enhanced visceral afferent transmission to the NTS [383]. Kirchgessner and her colleagues [384] provided the initial suggestion that this projection is involved in eating by showing that knife cuts that sever the paraventricular nucleus-hindbrain oxytocin projections resulted in hyperphagia and obesity. More recently, Blevins and colleagues [385, 386] confirmed this and also showed that reductions in hindbrain oxytocin signaling reduce the satiating potency of CCK in rats. Leptin also seems to affect this oxytocin signaling: (1) leptin activated paraventricular nucleus oxytocin neurons and increased the expression of oxytocin mRNA in the paraventricular nucleus, probably via melanocortin-dependent mechanisms [345, 350, 387, 388] and (2) third ventricular administration of oxytocin antagonists blunted leptin's anorectic effects and abolished its ability to enhance CCK activation of the NTS [350] as well as reversing the anorectic effect of α-MSH [389].

Conclusions

We have reviewed the basic physiology of satiation. Eating contributes to the regulation of energy homeostasis via control of satiation. Gastrointestinal nutrient sensing generates negative-feedback signals encoded as concentrations of the peptides CCK, GLP-1, and PYY (3–36) secreted during meals. There is substantial evidence that each of these contributes to the normal physiological control of satiation. Metabolic-sensing neurons located mainly in the hypothalamus and caudal brainstem react to circulating levels of glucose, FFA, leucine, leptin, and insulin to generate signals that may also contribute to satiation, but the physiological status of most of these is not yet firmly established. A number of neurotransmitters are involved in the processing of these signals into satiation. A main feature of this processing is that information derived from metabolic-sensing neurons converges on the caudal brainstem interneuronal networks that process information derived from gastrointestinal nutrient-sensing rather than projecting directly onto the motor neural networks that finally control eating. Progress in understanding these control of satiation should provide a platform for future research addressing the physiologies of the myriad other controls of satiation such as food reward, cognitive, and social controls. Furthermore, better understanding of the operation of this satiation system described here should suggest opportunities for novel peripheral and brain manipulations that may improve therapies for overeating and obesity and the many diseases that they lead to.

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