

Review

# Hypothalamic control of energy balance: different peptides, different functions<sup>☆</sup>

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## Abstract

Energy balance is maintained via a homeostatic system involving both the brain and the periphery. A key component of this system is the hypothalamus. Over the past two decades, major advances have been made in identifying an increasing number of peptides within the hypothalamus that contribute to the process of energy homeostasis. Under stable conditions, equilibrium exists between anabolic peptides that stimulate feeding behavior, as well as decrease energy expenditure and lipid utilization in favor of fat storage, and catabolic peptides that attenuate food intake, while stimulating sympathetic nervous system (SNS) activity and restricting fat deposition by increasing lipid metabolism. The equilibrium between these neuropeptides is dynamic in nature. It shifts across the day–night cycle and from day to day and also in response to dietary challenges as well as peripheral energy stores. These shifts occur in close relation to circulating levels of the hormones, leptin, insulin, ghrelin and corticosterone, and also the nutrients, glucose and lipids. These circulating factors together with neural processes are primary signals relaying information regarding the availability of fuels needed for current cellular demand, in addition to the level of stored fuels needed for long-term use. Together, these signals have profound impact on the expression and production of neuropeptides that, in turn, initiate the appropriate anabolic or catabolic responses for restoring equilibrium. In this review, we summarize the evidence obtained on nine peptides in the hypothalamus that have emerged as key players in this process. Data from behavioral, physiological, pharmacological and genetic studies are described and consolidated in an attempt to formulate a clear statement on the underlying function of each of these peptides and also on how they work together to create and maintain energy homeostasis.

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**Keywords:** Neuropeptide Y; Agouti-related protein; Melanin-concentrating hormone; Galanin; Orexins; Melanocortins; Galanin-like peptide; Cocaine- and amphetamine-regulated transcript; Corticotropin-releasing factor; Obesity; Diet

## 1. Introduction

I am honored to be invited to write this review, celebrating the 25th Anniversary of the journal, *Peptides*. This invitation was based on four of my earlier papers published in *Peptides*, on the role of neuropeptides in feeding and obesity, which became some of the most cited papers published in this journal over the past 25 years. The most frequently cited of these four papers was published in 1986 with Glenn Stanley, then a postdoctoral fellow, as first author [389]. This article demonstrated that repeated injections of neuropeptide Y (NPY) into the paraventricular nucleus (PVN) of rats cause a marked increase in daily food consumption and body weight. Another of my frequently cited papers, published in 1982 with a graduate student Linda Hor, demonstrated that injection of the opioid peptide,  $\beta$ -endorphin, into the

PVN also stimulated feeding, perhaps working in conjunction with norepinephrine [228]. The two other frequently cited papers, published in 1985 and 1992 with Glenn Stanley, further characterized the NPY-induced feeding response and showed that NPY stimulates the ingestion of carbohydrate [388] and that NPY acts through a variant of the Y1 receptor subtype [391].

These papers were published at an exciting period in the field of neuroscience. At this point in time, the idea that peptides in the brain could have a neuromodulatory function was just beginning to surface. Also, there was little understanding of the brain's role in body weight regulation and obesity. Thus, the possibility suggested by these four papers, that peptides in the hypothalamus are actively involved in the control of eating, nutrient balance and body weight, is largely the reason for their frequent citation. They were the start of an era that led in the 1990s to an explosion of research and to many remarkable accomplishments in this field of study. In this invited review, it is my honor and privilege to summarize this body of research and show the

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### Endorphinergic and $\alpha$ -noradrenergic systems in the paraventricular nucleus: Effects on eating behavior

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#### Abstract

Brain cannulated rats were injected with the opioid peptide  $\beta$ -endorphin ( $\beta$ -EP) directly into the hypothalamic paraventricular nucleus (PVN) where norepinephrine (NE) is most effective in stimulating eating behavior.  $\beta$ -Endorphin (1.0 nmole) reliably increased food intake in satiated animals, and this response was blocked by local administration of the selective opiate antagonist naloxone. The eating induced by  $\beta$ -EP was positively correlated in magnitude with the NE response and, like NE, was antagonized by PVN injection of the  $\alpha$ -noradrenergic blocker phentolamine. Naloxone had no effect on NE-induced eating, and the dopaminergic blocker fluphenazine failed to alter either  $\beta$ -EP or NE eating. When injected simultaneously, at maximally effective doses,  $\beta$ -EP and NE produced an eating response which was significantly larger than either of the responses elicited separately by  $\beta$ -EP or NE and was essentially equal to the sum of these two responses. The evidence obtained in this study suggests that  $\beta$ -EP and NE stimulate food ingestion through their action on PVN opiate and  $\alpha$ -noradrenergic receptors, respectively, and that  $\beta$ -EP's action is closely related to, and in part may be dependent upon, the PVN  $\alpha$ -noradrenergic system for feeding control.

**Keywords:** Norepinephrine;  $\beta$ -Endorphin;  $\alpha$ -Noradrenergic receptors; Opiate receptors; Naloxone; Feeding behavior; Neuropeptides; Hypothalamus; Paraventricular nucleus

many advances that have been made in our understanding of how neuropeptides control energy homeostasis. To join me in preparing this review, I have asked Katherine Wortley, a recent postdoctoral fellow in this lab who has published two important papers in this area [452,453].

In this review, we have chosen to focus on nine peptides that have received the greatest attention over the past 20 years. These include five peptides that stimulate feeding behavior, namely, NPY, agouti-related protein (AgRP), melanin-concentrating hormone (MCH), galanin (GAL), and the orexins, and four peptides that act to suppress feeding, namely, pro-opiomelanocortin (POMC), galanin-like peptide (GALP), cocaine- and amphetamine-regulated transcript (CART), and corticotropin-releasing factor (CRF).

The information accumulated on each of these peptide systems is organized in seven different sections related to: (1) the anatomical distribution of cell bodies, terminals and receptors; (2) endocrine signals that control its endogenous expression patterns, including hormones that indicate energy abundance (insulin and leptin) or energy insufficiency (ghrelin and corticosterone); (3) signals related to diet and circulating nutrients, glucose and lipids, that also modulate the endogenous peptide; (4) behavioral and metabolic effects induced by central injections of the peptide; (5) changes resulting from genetic mutations of the peptide; (6) the relationship of the peptide to other systems involved in feeding and body weight regulation; and (7) a concluding statement summarizing possible key functions of the peptide system. The evidence is fascinating in showing considerable redundancy in the different peptides, as well as great diversity and specialization in their actions. Both redundancy and diversity allow these peptide systems to be activated under a variety of physiological states and environmental conditions that occur over a lifetime and to function successfully in maintaining energy and nutrient homeostasis. In this review, we summarize a large body of information and formulate specific conclusions suggesting how feeding behavior and body weight are regulated, or deregulated, under these different conditions. The main conclusions drawn from this literature are schematically diagrammed in Fig. 1.

## 2. Peptides that stimulate eating and body weight

### 2.1. Neuropeptide Y

Neuropeptide Y (NPY), first isolated in 1982 [403,404], is a 36-amino acid member of a highly conserved group of peptides that are abundantly expressed in the hypothalamus. This peptide, a potent stimulant of feeding [81], is expressed in neurons of the arcuate nucleus (ARC) that send their dense projections to multiple areas of the hypothalamus. These include the PVN, ventromedial hypothalamus (VMH), and perifornical area/lateral hypothalamus (PFLH). It acts through different receptor subtypes, Y1R, Y5R and possibly Y2R, to affect energy homeostasis [187,410,445].

Neurons synthesizing NPY in the ARC are highly responsive to states of energy deficiency and higher metabolic demand [173,187,227,451]. Their expression is stimulated by food deprivation, as well as during states of increased exercise, cold and pregnancy. Elevated NPY expression is similarly seen after administration of the adrenal steroid, corticosterone (CORT), which is released in states of negative energy balance and performs a function of maintaining glucose availability. This steroid acts through glucocorticoid type II receptors to stimulate NPY gene expression, peptide synthesis, receptor activity, and NPY-induced feeding [12,268,390,407]. It exhibits a strong, circadian peak at the onset of the natural feeding period, when NPY expression and synthesis rise, and gluconeogenesis and glycogenoly-

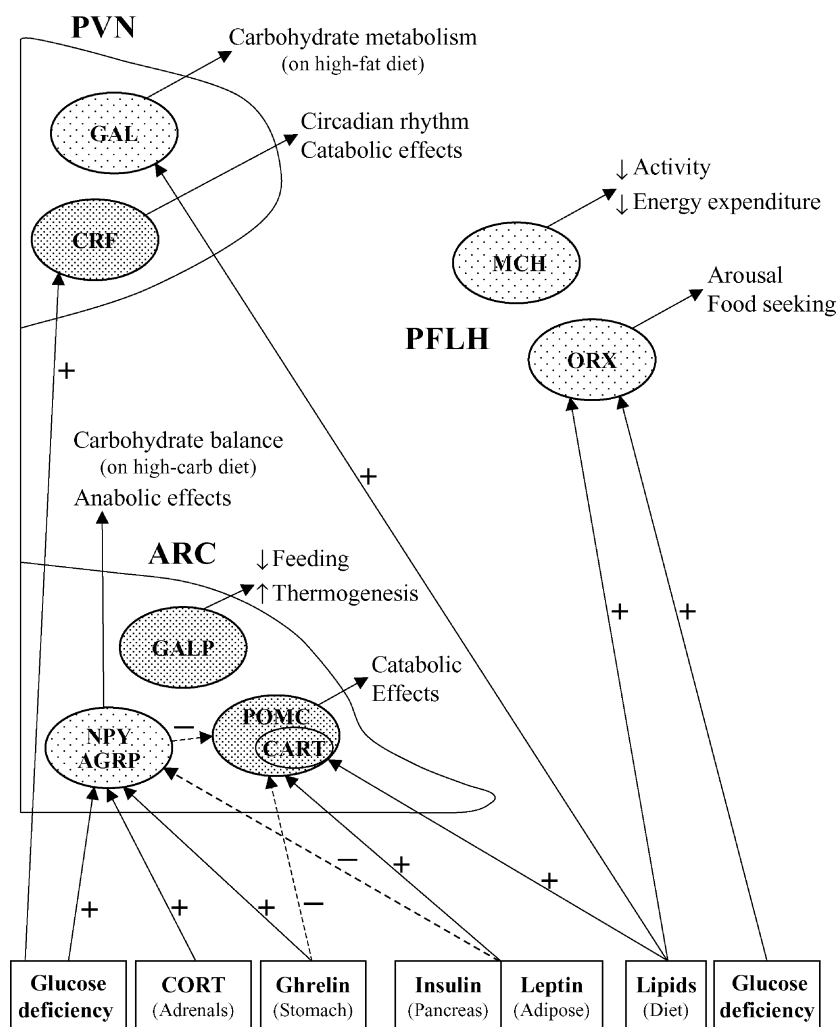


Fig. 1. Hypothesized model of nine peptide systems in the hypothalamus involved in energy homeostasis. Signals shown at the bottom, which control the expression and production of these peptides, are indicated with arrows as a stimulatory effect (+) or inhibitory effect (-). The behavioral and physiological actions of these peptides are also shown. Hypothalamic areas represented are the paraventricular nucleus (PVN), perifornical area + lateral hypothalamus (PFLH), and arcuate nucleus (ARC). The nine peptides are: AgRP, agouti-related protein; CART, cocaine- and amphetamine-regulated transcript; CRF, corticotropin-releasing factor; GAL, galanin; GALP, galanin-like peptide; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; ORX, orexins; and POMC, pro-opiomelanocortin.

sis are elevated to support the utilization of carbohydrate [9,224,374,407].

Similar to CORT, the hormone, ghrelin, an endogenous ligand of the growth hormone secretagogue receptor (GHS-R) that is released primarily from the stomach [198], is an indicator of energy insufficiency that may affect NPY expression. Levels of this hormone rise during fasting or before meal consumption, and they decline after eating or with high-fat diet consumption and obesity [15,18,31,93,416,418]. Also, ghrelin levels and GHS-R mRNA in the ARC are reduced in juvenile, obesity-prone rats [237]. A possible role for ghrelin acting through brain GHS-R to reverse states of negative energy balance is suggested by evidence that central ghrelin administration increases food consumption and c-Fos immunoreactivity in the PVN, a marker of neuronal activation, and it reverses

anorexia induced by leptin [304,372]. Also, chronic administration of this hormone, either centrally and peripherally, stimulates food intake and increases weight gain and fat mass by decreasing lipid utilization [289,416,417,456]. A relationship of ghrelin to NPY is demonstrated by the finding that this hormone stimulates food intake most markedly when injected directly into the ARC [455], and it induces Fos expression most predominantly in NPY neurons of the ARC [289,436], where the GHS-R mRNA is expressed [137]. Also, ghrelin stimulates the activity of these neurons [89] and increases NPY expression in the ARC, while antibodies and antagonists of NPY or ablation of the ARC block ghrelin's orexigenic effect [289,400]. Whereas these results indicate that NPY may have a role in mediating ghrelin-induced feeding, the evidence that NPY knockout mice respond normally to a ghrelin receptor agonist

suggests that other peptide systems are also likely to be involved [417].

These positive relations between NPY and CORT or ghrelin contrast with the inverse relationships detected with the adiposity-related hormones, insulin and leptin. These hormones are both reduced in states of negative energy balance, such as food deprivation, and they rise in close relation to body fat, providing a signal of energy abundance [24,318]. They gain access to the brain through saturable transport mechanisms and act as humoral feedback regulators via specific receptors to modulate food intake and energy balance. Whereas these hormones are likely to function at multiple sites in the brain as well as the body, investigators have focused attention on the ARC, where insulin and leptin receptors and their intracellular signaling molecules are concentrated and the hormones act upon the insulin-receptor-substrate phosphatidylinositol 3-kinase pathway [24,296]. The importance of these hormones as adiposity signals to the brain is revealed by evidence that central injections of leptin or insulin, or hyperleptinemia produced by an adenovirus gene transfer of the *ob* gene, reduce food intake, meal size and weight gain and stimulate energy expenditure and sympathetic nervous system (SNS) activity [24,99,451]. Also, insulin- or leptin-deficient animals are hyperphagic and obese, effects reversed by hormone administration. A close relationship to NPY is indicated by studies showing that injections of insulin and leptin reduce NPY gene expression, peptide levels and NPY-stimulated feeding [3,186,360,435]. This effect may be mediated by leptin and insulin receptors on NPY neurons [318] and may involve a direct, inhibitory effect of these hormones on the firing of NPY neurons [88]. It may contribute to the reduction in food intake and body weight induced by these hormones, as well as explain the enhanced NPY expression seen after food deprivation [185,451] and in normal-weight obesity-prone rats that are less sensitive to the inhibitory effect of leptin on NPY [235,236]. Collectively, these findings illustrate the homeostatic principle of negative feedback, whereby a rise in insulin and leptin with food consumption and weight gain suppresses the expression of anabolic peptides like NPY that would promote further weight gain.

In addition to these hormones, there is evidence that dietary nutrients, as well as circulating and even intracellular nutrients, can affect the expression of peptides involved in eating behavior, shunting fuels from short-term availability to long-term storage [298,362]. A variety of evidence suggests that NPY is closely related to glucose and its metabolism, as well as to dietary carbohydrate. In chronic studies with rats given a single diet, the expression of NPY in the ARC is consistently higher in subjects on a low-energy, high-carbohydrate diet compared to a high-fat diet, with the change more closely related to the rise in carbohydrate rather than the decline in fat [128,432,441]. Whereas these chronic, single-diet studies link endogenous NPY to dietary carbohydrate, further measurements of cir-

culating hormones and nutrients suggest that the elevated NPY is more likely attributed to the reduction in leptin or rise in CORT on the low-energy diet, rather than to any change in the levels or metabolism of glucose [432]. Other studies allowing subjects to exhibit their natural choice of dietary macronutrients are more informative and provide evidence relating endogenous NPY to voluntary selection of carbohydrate. For example, NPY mRNA and peptide immunoreactivity in the ARC are found to peak at the onset of the natural feeding cycle, when carbohydrate is the highly preferred macronutrient and carbohydrate stores are low [9,179,374]. They also rise dramatically around the time of weaning [184], when rats exhibit a strong preference for carbohydrate and have minimal body fat [230]. Further, NPY peptide levels prior to the start of the active feeding period are strongly, positively correlated with the amount of carbohydrate selected voluntarily by normal-weight rats with similar levels of leptin [33,179]. They are unrelated, however, to shifts in fat consumption at this time, consistent with recent evidence that NPY expression is unaffected by administration of Intralipid, which elevates circulating lipids [70]. Thus, endogenous NPY is positively linked to an animal's voluntary selection of carbohydrate, which is likely to reflect a deficiency of carbohydrate stores.

This relationship linking NPY to a need for carbohydrate suggests that this peptide may be related to signals reflecting a deficiency of glucose availability, stores or utilization. This is supported by evidence that NPY mRNA levels in the ARC are significantly elevated after pharmacological blockade of glucose utilization with 2-deoxy-D-glucose (2-DG) [13,365,366], which produces no change in circulating leptin [22], and they are increased just prior to the onset of the active feeding cycle, when glucose levels and stores are declining [9,407]. Further, NPY expression is markedly affected by acute administration of glucose, showing an inverse relation to circulating levels. Within the first 30–60 min after glucose injection, NPY is initially suppressed as glucose levels are significantly elevated; after 90 min, however, this effect is reversed, leading to a stimulation of NPY as glucose levels drop to baseline [71,434]. These shifts in NPY, which occur in the absence of changes in leptin, are consistent with a physiological role for NPY in stimulating spontaneous meals, which in animals and humans are invariably preceded by a decline in glucose 10–30 min before meal initiation [60,61]. Thus, together with the above studies of carbohydrate selection, these results establish a close relationship of NPY to signals reflecting disturbances in glucose levels and glucose utilization.

A mechanism mediating these changes is suggested by evidence that glucokinase, a sensor of glucose utilization, is expressed in NPY neurons of the ARC [255]. These NPY neurons are found to be “glucose sensitive,” excited by a decline in glucose levels and glucose utilization [13,283], possibly through the  $\text{Na}^+ - \text{K}^+ - \text{ATP}$  pump or ATP-activated  $\text{K}^+$  channel [238]. The intracellular processes in the hypothalamus that signal changes in glucose availability may

involve the enzyme, malonyl coenzyme A (CoA) [344], which switches substrate oxidation from lipids to excess glucose, and also the hexosamine biosynthetic pathway, which is activated by glucose flux [299]. A role for malonyl CoA is suggested by evidence that this enzyme is reduced in states of energy deficiency [344] that stimulate NPY in the ARC, and it is elevated by glucose [346] that reduces NPY expression. It is also increased by C75, a compound that enhances glucose utilization while inhibiting fatty acid synthase and that suppresses NPY gene expression [249,371,454]. Thus, in states of reduced glucose stores and utilization and a physiological need for dietary carbohydrate, a decline in intracellular malonyl CoA may provide an important signal for stimulating NPY expression, acting either directly or indirectly through its effects on glucose utilization.

Consistent with these investigations of endogenous NPY is evidence from central injection studies suggesting a role for NPY, in states of negative energy balance, in stimulating the ingestion and metabolism of carbohydrate, to maintain glucose homeostasis while promoting fat synthesis with available carbohydrate. These actions of exogenous NPY [39,187,221,224,337,386], include a potent, stimulatory effect on feeding behavior, with a preferential increase in the ingestion of carbohydrate under conditions when this macronutrient is preferred or needed [224,239,388,408,440]. This suggests a positive feedback loop, whereby this peptide's immediate function is not only to replenish carbohydrate stores but to generate excess carbohydrate that may be geared towards fat storage. This is supported by evidence that NPY injection stimulates the release of both CORT, which controls circulating glucose levels, and insulin [221,231,349], which function together to promote de novo lipogenesis [163,274]. Injection of NPY produces an increase in respiratory quotient, which reflects reduced fat utilization in favor of carbohydrate [270]. Further, repeated NPY injections stimulate carbohydrate intake more than fat intake and produce a greater increase in body fat accrual, along with metabolic disturbances, than would be expected based on the elevated food intake alone [177,329,387,389]. Exogenous ghrelin, which stimulates NPY neurons, similarly increases food intake and respiratory quotient [416,417], suggesting the involvement of NPY in its actions. Moreover, acute injections of NPY antisera and receptor antagonists invariably reduce food intake [187,233,391], while chronic antisense oligodeoxynucleotides to NPY mRNA suppress endogenous NPY production and weight gain [10]. The NPY-induced metabolic changes, reflecting enhanced parasympathetic nervous system activity and reduced sympathetic activation, include a reduction in energy expenditure and thermogenesis, decreased glucose uptake in muscle, and a diversion of excess energy and glucose towards fat synthesis in white adipose tissue [110,308].

In light of the above evidence, the results obtained in NPY or NPY receptor transgenic and knockout mice models are somewhat surprising [160,307,410]. The NPY knockout and overexpressing mice show little change in

baseline eating or body weight [116,173,325,410]. Also, mice deficient in the Y1 or Y5 receptors, through which NPY may act to promote hyperphagia and weight gain, actually exhibit a mild form of obesity [210,262]. While these patterns may be explained by a compensatory rise in other feeding-stimulatory peptides in these mutant mice [227], there is additional evidence for more specific changes in NPY or Y1 receptor-deficient mice that are consistent with this peptide's known actions in states of negative energy balance. This phenotype includes a decrease in fasting-induced refeeding [21,364], diabetes-induced hyperphagia [376], and weight gain in leptin-deficient, *ob/ob* mice [117,321], and a reduction in respiratory quotient, reflecting decreased utilization of carbohydrate relative to lipids as a source of oxidizable substrates [364]. Also, mice deficient in the Y1 receptor show a marked reduction in refeeding after a fast [311,312], while mice with NPY overexpression exhibit an obese phenotype accompanied by overeating, hyperglycemia, and hyperinsulinemia, as well as with increased weight gain and Y1 receptor expression [183]. This obese phenotype in NPY transgenic mice is evident only on a sucrose-loaded diet [183], consistent with the proposed role for NPY in gearing excess carbohydrate substrates towards lipogenesis. This is in contrast to a fat-rich diet, which reveals no effect on body weight or food intake in NPY knockout mice compared to wildtype controls [165]. The recent finding in Y2/Y4 double knockout mice of a markedly lean phenotype and reduced brown fat mass, despite significant hyperphagia, suggests a possible function for these receptor subtypes in energy metabolism [348].

These effects of NPY are likely to involve connections to other neural systems involved in energy homeostasis [186,187,445]. These may include reciprocal connections with systems that inhibit feeding, such as, the melanocortins in the ARC, CRF in the PVN, and serotonin (5-HT) in the midbrain. There is evidence that NPY axons colocalizing  $\gamma$ -amino butyric acid (GABA) project to and inhibit POMC cells of the ARC [88], indicating that NPY may produce its anabolic effects by suppressing the melanocortin system. Further connections may also involve peptides that stimulate feeding, including the orexins and MCH in the PFLH [187]. The demonstration that NPY neurons in the ARC co-express the peptide, AgRP, an endogenous antagonist of the feeding-inhibitory melanocortin system, suggests another avenue of communication between NPY and other known peptides controlling feeding [51,77,445].

In summary, these investigations demonstrate that NPY neurons in the ARC are stimulated under conditions of negative energy balance, including after food deprivation, on a low-energy diet, before the onset of the natural feeding cycle, or in weanling animals with little body fat. As shown in Fig. 1, this peptide rises in response to changes in various hormones, including elevated levels of glucocorticoids and ghrelin and reduced levels of leptin and insulin. It also responds positively to signals related to a need for carbohydrate, decline in glucose levels, or disturbance in glucose

utilization. The function of endogenous NPY during food scarcity or in states of low carbohydrate stores is to promote food intake, reduce fat oxidation in favor of carbohydrate, and stimulate de novo lipogenesis. These NPY effects are facilitated by local GABA neurons that simultaneously reduce the firing of melanocortin neurons in the ARC and their catabolic actions.

## 2.2. *Agouti-related protein*

The peptide, agouti-related protein (AgRP), is expressed primarily in the ARC [51,142], in neurons that also produce NPY [25,51]. It forms part of the central melanocortin system (see below), which comprises  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH), an agonist of both the melanocortin receptor-3 (MC3-R) and melanocortin receptor-4 (MC4-R), in addition to AgRP, an endogenous, competitive antagonist of these receptors [65,119,150,293,303,339]. This peptide was discovered by its homology to the agouti protein, which is expressed in the skin and regulates coat pigmentation in rodents. Although the C-terminal region of agouti and AgRP, which are sufficient for melanocortin receptor binding, are approximately 40% identical, they bind to distinct sets of melanocortin receptors, with agouti binding to MC1-R and MC4-R and AgRP binding to MC3-R and MC4-R [303,461]. Emerging evidence suggests that, in addition to antagonizing the effect of  $\alpha$ -MSH at the MC3-R and MC4-R, AgRP suppresses the basal activity of the MC4-R, which characterizes this peptide as an inverse agonist [150]. Whereas immunohistochemical studies show that AgRP cell bodies are restricted to the ARC, AgRP fibers project to many brain areas and multiple areas within the hypothalamus, including the PVN and PFLH, and all of these AgRP terminals contain NPY [25,51].

Studies of endogenous peptides reveal a close functional relationship between AgRP and the NPY neurocircuit. In addition to coexisting neurons of the ARC, the endogenous expression of these peptides is similarly modulated under various physiological conditions [275,466]. This is evident in states of negative energy balance or increased energy demand, such as food deprivation or during lactation, which have reduced leptin and insulin levels and increased ghrelin or CORT levels and which stimulate AgRP together with NPY [77,142,275]. Also, AgRP like NPY is suppressed by ADX and stimulated by CORT replacement [16,256,354], and it shows a strong circadian rhythm, rising with CORT at the onset of the nocturnal feeding cycle [252]. Conversely, both AgRP and NPY are suppressed under conditions of positive energy balance, when leptin and insulin levels are elevated and ghrelin levels are reduced. This is revealed in obese animals compared to lean subjects or in animals injected with leptin [108,466], and it is also seen after insulin injection in streptozotocin-induced diabetic rats [327].

Results obtained with manipulations of diet and circulating nutrients and their metabolism reveal further similarities between these two peptides. As with NPY, AgRP gene ex-

pression is higher in rats on a low-energy diet compared to a fat-rich diet [431,466]. Also, it is increased under conditions where glucose utilization is reduced, such as after injection of 2-DG [366] or in streptozotocin-induced diabetes [327]. Recent studies in this lab demonstrate that endogenous AgRP similar to NPY is suppressed by a single injection of glucose, while this effect is subsequently reversed into a stimulation as glucose levels decline toward baseline [71]. These responses to changes in glucose contrast with the lack of change in AgRP after administration of Intralipid, which increases circulating lipids [70]. Thus, AgRP shows a clear, inverse relation to glucose levels and metabolism.

As with NPY, central injection of AgRP has a potent stimulatory effect on food intake [141], similar to the response induced by a synthetic melanocortin receptor antagonist [342]. This stimulatory effect of a single AgRP injection, apparent within 4 h of administration, lasts up to 6 days [141,342], a longer duration than that observed with NPY. This long-term effect does not involve the MC4-R [141], suggesting that AgRP may produce long-term changes to the neural pathways downstream of this receptor. Chronic administration of AgRP, like NPY, also increases daily food intake and decreases oxygen consumption and the capacity of brown adipose tissue to expend energy, effects which lead to increased body fat accrual and leptin levels [380,381]. While these peptides have somewhat different effects on c-Fos immunoreactivity in extra-hypothalamic areas [140,449], they are additive in their effects on feeding [449] and are suggested to send similar types of signals that reinforce one another, possibly through the inhibition of a Gs-coupled receptor by AgRP and the activation of a Gi-coupled receptor by NPY [448]. Both peptides appear to mediate the orexigenic effect of ghrelin [289]. Thus, they may represent redundant components of a parallel circuit, possibly explaining why mice with a deficiency of NPY but an intact melanocortin system exhibit only minor defects in their eating behavior and leptin signaling [116].

In genetic studies, the overexpression of the *Agrp* gene in transgenic mice produces a phenotype similar to NPY. The mice exhibit hyperphagia and obesity, in addition to increased body length, hyperinsulinemia, late-onset hyperglycemia, and pancreatic-islet hyperplasia [132,303]. These features are similar to those seen in mice that ectopically express the *agouti* gene [52,195], as well as in MC4-R-deficient mice [170]. However, AgRP-deficient mice, similar to NPY-deficient or AgRP and NPY double knockout mice, are normophagic and exhibit normal body weight and body composition under ad libitum feeding conditions [325], indicating that these anabolic peptides in the ARC do not function tonically when food is freely available. They do act, however, under conditions where food is scarce. This is revealed by the finding that AgRP knockout mice, like NPY mutants, have impaired reflexive hyperphagia in response to a 24-h fast [378]. This supports the physiological data implicating AgRP in processes geared towards restoring energy homeostasis when food

sources are low. This peptide may act in balance with the melanocortin  $\alpha$ -MSH at the MC4-R but may also affect additional targets besides this receptor [263].

The idea of an antagonism between the AgRP/NPY system and the melanocortins in controlling eating and body weight is strengthened by evidence showing changes in endogenous AgRP that are opposite to those seen with the melanocortin peptides. Electrophysiological studies suggest that AgRP neurons interact with POMC neurons in the ARC through GABA. AgRP/NPY axons that colocalize GABA project onto ARC POMC cells and inhibit their neural activity [88], supporting the idea that AgRP/NPY neurons produce anabolic effects by suppressing the melanocortin system. Circulating factors impacting on the ARC may act indirectly through these local GABA-containing AgRP/NPY neurons to affect POMC cell firing. One such example is leptin, which inhibits the firing of AgRP/NPY neurons and reduces the release of GABA onto POMC neurons, leading to their disinhibition [88]. Conversely, ghrelin stimulates NPY/AgRP neurons, inducing an increase in GABA release and inhibition of POMC neurons [89]. This neurocircuit leads one to expect effects of AgRP, as well as NPY, that are opposite in nature to those induced by the melanocortins. This is generally found to be the case, as described below.

Taken together, this evidence demonstrates that AgRP in the ARC is similar to NPY in being stimulated by conditions of energy deficiency, which involve increased levels of ghrelin and CORT and reduced levels of leptin and insulin (Fig. 1). This endocrine profile is evident in subjects at the onset of the active phase, after food deprivation, in states of low glucose utilization, or on low-energy diets. Under these conditions, increased production of AgRP is required to stimulate food intake, reduce lipid oxidation in favor of carbohydrate, and promote fat synthesis. The effects mediated by these peptides are facilitated by local GABA neurons, which reduce the firing of melanocortin neurons in the ARC that induce catabolic effects. These two parallel systems, AgRP and NPY, which are activated under similar conditions and have comparable effects, very likely evolved to ensure the signaling of hunger during food scarcity and to enable the body to endure long periods of negative energy balance.

### 2.3. Melanin-concentrating hormone

There exist neurons in the area of the PFLH and zona incerta that synthesize the orexigenic peptide, MCH, a cyclic 19-amino acid neuropeptide originally isolated from salmon pituitaries [288,423]. This peptide acts via a specific receptor, MCHR1, which has a typical seven transmembrane domain region seen with G-protein coupled receptors [66,350]. Whereas the peptide is expressed predominantly in the PFLH region [317], the MCHR1 is broadly expressed [158,350]. It is evident in the ARC and VMH, where it may have a role in feeding and energy homeostasis, but it is more heavily expressed in extrahypothalamic areas, which process olfactory information and are involved in reward-

ing aspects of motivated behaviors. The specific function of this peptide remained obscure until the discovery, with RT-PCR differential display, that MCH is overexpressed in leptin-deficient *ob/ob* mice [326], suggesting a role in body weight regulation.

Studies of endogenous MCH in the PFLH provide support for this peptide's role in feeding and body weight regulation [191,317]. The expression of MCH is stimulated by fasting [326]. Although fasting reduces leptin levels and leptin injection suppresses MCH as well as MCHR1 mRNA [38,199,347], this fasting-induced increase in MCH expression can be seen in *ob/ob* mice with leptin deficiency, under ad libitum feeding or fasted conditions [326]. Also, rats that become obese on a fat-rich diet and have markedly elevated levels of leptin show no difference in MCH mRNA compared to lean rats [125,412]. This indicates that MCH expression is regulated by other factors in addition to leptin. Consistent with this idea, MCH gene expression is responsive to glucocorticoid status, reduced in ADX rats and stimulated by dexamethasone [322]. It is also responsive to E<sub>2</sub>, which suppresses MCH mRNA in ovariectomized animals and blocks the deprivation-induced increase in MCH [284,285]. Similar to the effects of fasting, MCH is increased by exposure to a cold environment [314] and also by injection of  $\beta_3$ -adrenergic receptor agonists that cause anorexia [284,285].

There is limited evidence regarding the sensitivity of MCH neurons to dietary or circulating nutrients. This peptide has yet to be tested with respect to the effects of a high-fat or high-carbohydrate diet on gene expression. Nor has it been linked to changes in circulating levels of glucose or lipids. The only available evidence indicates that MCH responds to a deficiency in fat oxidation or synthesis but is insensitive to changes in glucose availability or its utilization. The expression of MCH in the PFLH is stimulated by administration of the fatty acid oxidation inhibitor, mercaptoacetate [365,366], or the fatty acid synthase inhibitor, C75 [371]. It is unresponsive, however, to injection of 2-DG, as well as of goldtioglucose, a toxic form of glucose [26,28,134,365].

These results obtained with measurements of endogenous MCH reveal clear differences between this peptide in the PFLH and the peptides in the ARC. Whereas MCH is similar to NPY and AgRP in being stimulated by food deprivation and inhibited by leptin, it differs in being unaffected by ghrelin administration [413] and stimulated by insulin [19] or the fatty acid synthase inhibitor, C75 [314,371], which have a potent, inhibitory effect on NPY/AgRP mRNA. Also, MCH expression remains unaltered in obese compared to lean rats on a high-fat diet [125,412], which have reduced NPY and AgRP mRNA along with elevated insulin and leptin, and it is suppressed during pregnancy and lactation [126], conditions that invariably increase NPY mRNA. Further differences are evident in the failure of MCH to respond to a decline in glucose availability or utilization [26,28,134,365], which has a potent stimulatory effect on NPY, and in the increased MCH

expression after pharmacological blockade of fat oxidation [365,366], which has no impact on NPY. These marked differences between MCH and the ARC peptides suggest that they function under different physiological conditions, with MCH responsive to states of fatty acid deficiency.

The involvement of endogenous MCH in the control of feeding behavior and energy homeostasis is supported by the finding that central administration of this peptide potentiates feeding [326,341], an effect that occurs when injected into the ARC and PVN [1,340]. This MCH-induced feeding response, however, is relatively small and of short duration compared to that of NPY [109]. There is evidence that MCH may have a greater impact on metabolic processes that contribute to weight gain. This is indicated by the finding that blockade of MCH expression by antisense MCH oligonucleotide in cold-exposed rats, while having little impact on feeding, causes a loss in body weight together with an increase in brown fat mass and uncoupling protein 1 (UCP1) expression [314]. It also suppresses the release of the thyroid stimulating hormone [193], while enhancing the secretion of CORT [192]. In pharmacological studies with chronic MCH, an initial report with repeated ventricular injections of this peptide in rats showed little change in daily food intake and body weight [341]. However, subsequent studies with chronic infusions of MCH or MCHR1 agonist reveal hyperphagia, together with increased caloric efficiency, weight gain, lipogenic activity and body fat mass [98,174,369]. Also, the infusion of MCH in mice produces the hyperphagic obese phenotype on a high-fat diet more than a lab chow diet, in association with a decrease in body temperature and reduced fatty acid oxidation and thermogenesis in brown adipose tissue [130,174]. Conversely, chronic administration of an MCHR1 antagonist produces a decrease in body weight, due to both a reduction in food intake and increase in energy expenditure [121,369]. Together, these findings indicate that MCH acts through metabolic as well as behavioral mechanisms.

A physiological role for MCH in energy homeostasis is strongly supported by genetic studies. Transgenic mice that overexpress MCH in the PFLH, while maintaining normal weight and normal feeding on a standard diet, exhibit hyperphagia, hyperleptinemia, hyperglycemia and insulin resistance on a high-fat diet compared to wild-type animals, with the obesity possibly secondary to the hyperphagia [254]. Moreover, targeted ablation of the MCH gene leads to a thin phenotype, associated with hypophagia and an increase in metabolic rate [370], and it attenuates the marked obesity of *ob/ob* mice, through an increase in basal energy expenditure and locomotor activity level rather than food intake [363]. Mice lacking functional MCHR1 gain less weight than wild-type littermates on a high-fat diet, due to an increase in metabolic rate and thermogenesis that is secondary to increased locomotor activity [78,264]. The additional finding, that these mice become leaner despite a significant increase in food intake, supports the idea that MCH plays a more

important role in regulating energy expenditure and activity level than food intake.

While endogenous MCH clearly responds differently from the orexigenic peptides in the ARC in a variety of physiological conditions, there is evidence that it performs specific functions within the NPY signaling cascade. Investigations show reciprocal connections between NPY- and MCH-containing fibers of the ARC and PFLH [50], and chronic infusion of MCH activates NPY mRNA in the ARC [98]. Also, the MCH-induced feeding response is blocked by an NPY Y1 receptor antagonist [64], and it stimulates the release of NPY [1]. However, mice lacking the MCH or MCHR1 genes show no change in NPY gene expression [78,370], and combination injection studies consistently demonstrate additive effects of these two peptides on food intake at maximum doses [98]. Thus, the MCH feeding pathway may function in relation to other peptide systems in addition to NPY. Whereas chronic MCH administration has no impact on the expression of the orexins [98], these two peptide systems are anatomically associated in the PFLH, albeit in distinct neuronal populations [27], and they have reciprocal synaptic connections [136]. Further, they respond similarly in a positive direction to insulin and associated hypoglycemia [19,133,279] and in a negative direction to leptin [32,347]. Also, they are both reduced in pregnancy and lactation as well as in a pituitary grafted rat model of chronic hyperprolactinemia [126], although they show different patterns in obesity-prone animals on a high-fat diet, which compared to obesity-resistant subjects exhibit no change in MCH but a rise in orexin mRNA [412,453]. The similar location of these peptides in the PFLH has led investigators to suggest that they may both have a role in communicating the hedonic or rewarding aspects of the feeding process [100,353]. This postulate is countered, however, by the evidence that these lateral hypothalamic peptides stimulate food intake through different neural and behavioral circuits, with the orexins but not MCH dependent on the activation of opioid receptors and functionally active in stimulating the consumption of a fat-rich diet [82]. A relationship of MCH to the melanocortin system, acting in a downstream fashion, is suggested by evidence that neurons containing MCH in the PFLH receive projections from melanocortin neurons in the ARC [50,115] and that MCH production is subject to tonic inhibition from the melanocortins [145]. Also, MCH knockout mice exhibit a reduction in POMC expression [370], which may reflect a compensatory downregulation in the absence of MCH and may also contribute to the lean phenotype of this mutant.

Thus, these results suggest that MCH neurons in the PFLH have an important role in energy homeostasis (Fig. 1). Studies of MCH gene expression reveal similarities as well as differences to the orexigenic peptides of the ARC. MCH is similar to NPY and AgRP in being activated during states of negative energy balance and increased metabolic demand. It differs from these peptides, however, in functioning independently of leptin under certain conditions and being unresponsive to nutrient-sensing signals indicative of decreased

glucose availability and utilization. In restoring energy balance, it also differs in having a stronger effect on the output than input side of the equation, with a relatively small feeding response outweighed by its strong, anabolic effects that include a decrease in energy expenditure, activity level, fat oxidation and thermogenesis.

#### 2.4. Galanin

Galanin (GAL) is a 29-amino acid peptide that was originally isolated from the small intestine and is widely distributed throughout the hypothalamus and well conserved amongst species, including rats and humans [91,138,405]. It is expressed in a number of neuronal populations within the hypothalamus, including the PVN, its medial parvocellular and lateral magnocellular areas, the PFLH, ARC and other hypothalamic nuclei. These neurons send projections throughout the hypothalamus, where GAL receptor subtypes, GALR1 and GALR2, exist [138,221,408,457]. This peptide is suggested to have a causal role as a growth and prolactin-releasing factor to the lactotroph, especially in states of high estrogen exposure, and also as a neurotogenic factor in adult sensory neurons [457]. There is additional evidence, reviewed below and elsewhere [90,222,223], suggesting a role for GAL in feeding behavior and energy homeostasis.

Studies of endogenous GAL demonstrate marked differences between this peptide system and that of NPY and AgRP in terms of their responsiveness to endocrine and physiological signals. This is seen in their responses to the adrenal steroid, CORT, which has little impact on or transiently inhibits GAL gene expression in PVN neurons and has no effect on GAL's feeding response [11,154,407] but which has a potent stimulatory effect on NPY and AgRP and on NPY-induced feeding. Moreover, E<sub>2</sub> alone or in combination with P has a strong stimulatory effect on GAL in the PVN and median eminence [40,46,124,273], which is not seen with NPY in the ARC. Whereas insulin suppresses both GAL and NPY [401,435], these peptides in the ARC show markedly different responses to leptin. This hormone, while producing a strong inhibition of NPY gene expression and release, produces little or no change in basal GAL expression in the ARC, GAL release from hypothalamic explants, and GAL-induced CRF release, and it causes only a small suppression of PVN GAL mRNA [36,80,368]. This differential responsiveness to leptin, possibly due to the low concentration of leptin receptors on GAL neurons [80], may explain their different responses to food restriction, which reduces leptin levels and has little impact on or suppresses GAL mRNA [30,45,186,224], while markedly enhancing NPY gene expression. It may also be related to the additional finding that PVN GAL mRNA rises in obese rats which have elevated leptin [104,234,323], a physiological state associated with a suppression of NPY. As described below, differential responses of GAL and NPY are also found in relation to disturbances in nutrient metabolism, with GAL

suppressed by an inhibitor of fat oxidation that has no effect on NPY [257,433] and GAL unaffected by an antagonist of glucose oxidation that potentiates NPY [433]. These differences between the peptide systems suggest that they may function through distinct mechanisms and in different physiological states or conditions.

Marked differences between GAL and NPY are also seen in studies examining the effects of diet as well as anti-metabolites that block nutrient metabolism. One main finding demonstrated in a number of studies and animal models is that GAL gene expression and peptide production in the PVN, but not the ARC, are positively related to the amount of fat consumed [8,223,225,300]. This peptide is stimulated by a high-fat diet and rises indirectly in relation to body fat accrual on a high-fat diet, despite an increase in leptin. This is in contrast to NPY in the ARC, which is unaffected or reduced by fat consumption and declines in relation to body fat, presumably due to an increase in leptin. In recent studies, GAL in the PVN is shown to be strongly, positively related to circulating levels of triglycerides, in addition to measures of fat uptake and oxidation in muscle [226]. This relationship is robust, seen in different models of dietary obesity. Of particular note is the finding that it is also evident under conditions of acute exposure to a high-fat diet, for 1 day or even 2 h, indicating its independence of changes in body fat and leptin [226]. Fat-preferring, obesity-prone rats exhibit higher levels of GAL mRNA and production in the PVN [90] while NPY is reduced, and these rats are more responsive than obesity-resistant rats to the feeding-stimulatory effects of GAL but not NPY [246]. A direct relationship between GAL and the metabolism of fat is suggested by evidence that pharmacological blockade of fat oxidation reduces PVN GAL expression while suppressing fat intake [433], and it stimulates the expression of GALR1 in the PVN [131]. In contrast to NPY, GAL shows little change after injection of 2-DG [433]. These studies of endogenous GAL suggest that this peptide functions specifically under dietary conditions rich in fat and responds to signals related to the metabolism of fat rather than carbohydrate.

Injection studies reveal a number of effects of GAL on behavioral, endocrine and metabolic actions, which are mediated via distinct GAL receptor subtypes, GALR1 and GALR2 [138,211,221,408]. The first behavioral investigation of GAL revealed a stimulatory effect on feeding behavior [212]. As shown in subsequent studies, this response is smaller and of shorter duration than that induced by NPY [109,186], revealing further differences between these peptides. While GAL has little impact on an animal's macronutrient preference, whether for carbohydrate or fat, a variety of evidence links this peptide's feeding-stimulatory response to dietary fat, similar to the positive relation established for endogenous GAL. In contrast to NPY, GAL-elicited feeding is stronger and more prolonged in subjects maintained on a high-fat compared to low-fat diet or in subgroups or strains of rats that naturally prefer

fat, and it is greatly attenuated when fat is removed from the diet [8,23,223,287,406]. Further, GAL-induced but not NPY-induced feeding is suppressed by the pentapeptide, enterostatin, and also by the GAL antagonist, M40, both of which reduce the consumption of fat [85,196,229,244]. This effect of enterostatin is associated with increased c-Fos immunoreactivity in the PVN where GAL neurons are concentrated [287]. Also, repeated PVN injections of antisense oligonucleotides to GAL mRNA produce a marked reduction in fat ingestion and weight gain, in conjunction with a decline in PVN GAL levels [84,229]. Whereas both GAL and NPY reduce energy expenditure and inhibit SNS activity [269,286], GAL's endocrine and gastrointestinal effects are, in some cases, diametrically opposite to those of NPY [197,200,407]. They include a suppression of insulin, CORT and vasopressin release and an increase in gastric acid secretion.

While GAL has no effect on respiratory quotient [269] in contrast to the marked increase induced by NPY, other results from this lab [464] reveal an effect of GAL on carbohydrate and fat metabolism in muscle. Acute PVN injection of GAL, but not NPY, stimulates the activity of the enzyme phosphofructokinase in muscle, which reflects increased capacity to metabolize carbohydrate [375]. This response is accompanied by a decrease in the activity of  $\beta$ -hydroxyacyl-CoA dehydrogenase, reflecting a reduced capacity to metabolize fat. Since endogenous GAL and the GAL-induced feeding response are both stimulated by a high-fat diet and are elevated in animals selecting a fat-rich diet as described above, this finding that GAL favors carbohydrate over fat metabolism suggests that this peptide may be activated to compensate for or reverse the metabolic effects of a high-fat diet, which include a reduction in carbohydrate metabolism [291]. It may also contribute to the hyperphagia on a high-fat diet, which may result in part from a need to consume sufficient carbohydrate to replenish liver glycogen levels [306]. Consistent with this proposed role for GAL in maintaining carbohydrate balance on fat-rich diets is the finding that PVN GAL expression, together with the selection of dietary fat, are both reduced in animals treated with a pharmacological agent that blocks fat oxidation [433].

The consequences of excess GAL stimulation may be seen under specific conditions. Studies published to date indicate that chronic ventricular infusion of GAL has little effect on daily food intake and weight gain in rats [383]. Whereas this negative result may be due to a variety of factors including the activation of compensatory mechanisms for maintaining body weight, there is evidence that it reflects, in part, desensitization to the effects of repeated GAL injection and also the lack of sufficient dietary fat to reveal GAL's actions. A significant increase in food intake and body weight can be seen with chronic GAL injections specifically in mice that are deficient in endogenous GAL [164]. Moreover, GAL injected directly into the PVN can significantly increase body fat accrual, leptin levels and adipose lipoprotein lipase in rats on a high-fat diet but not in subjects on moderate- or

low-fat diets [464]. Whereas endogenous GAL is invariably stimulated in rats that become obese on a high-fat diet [30,225,310], it is noteworthy that this peptide shows no change in rats that develop obesity on a high-carbohydrate diet [104]. This evidence further supports the idea that GAL functions to restore nutrient balance only under dietary conditions with a high-fat content.

To date, studies with GAL mutants indicate that mice with deletions of the GAL gene or GAL receptor genes or that overexpress GAL are able to defend their food intake and body weight when fed ad libitum on a low-fat diet or even fasted on this diet [91,457]. Whereas this suggests that GAL may not have a major role under these conditions, this lack of response in GAL mutants, as with animals receiving chronic GAL injections, may be attributed to other factors, such as the lack of sufficient dietary fat or the activation of compensatory mechanisms that help maintain nutrient homeostasis. Since GAL is believed to function specifically when dietary fat is in excess, GAL mutants may exhibit disturbances in feeding and body fat accrual only when maintained on a high-fat diet or when given diets allowing animals to express their natural preferences for fat as compared to carbohydrate or protein. Studies to date have only examined GAL mutants on standard lab chow diets. As for the activation of compensatory mechanisms, the evidence obtained so far in GAL knockouts shows increased sensitivity to the inhibitory effects of chronic leptin treatment on body weight and fat mass, suggesting that endogenous GAL plays a role in counteracting these actions [164]. These mice also exhibit disturbances in leptin-induced release of CORT during fasting, indicating disturbances in neuroendocrine homeostasis under stressful metabolic conditions.

To perform its functions of stimulating feeding and maintaining carbohydrate balance, there is evidence that GAL may act through other peptide systems. The opioids are thought to have some role in mediating GAL-induced feeding, as suggested by immunohistochemical results and the finding that naloxone attenuates the GAL feeding response [105]. Another study suggests that the stimulation of food intake by GAL may also involve the release of endogenous NPY [36]. Further evidence focuses attention on a relationship of GAL to the melanocortins, which as described below inhibit feeding on a high-fat diet and stimulate the oxidation of fat. A possible inhibitory effect of GAL on the melanocortins is suggested by evidence that POMC neurons in the ARC are innervated by GAL fibers [168] and possess GALR1 receptor mRNA [42]. Also, GAL has direct inhibitory action on ARC neurons expressing the GALR1 mRNA [320], and it specifically modulates the secretory activity of POMC neurons [41]. A corollary of this hypothesis links GAL to leptin, a potent stimulant of the melanocortins [6,409]. The possibility that GAL counteracts leptin's stimulatory effects on fat metabolism is supported by the finding that GAL knockout compared to wildtype control mice show increased sensitivity to the inhibitory effects of chronic leptin treatment on body fat accrual [164].

The decrease in fat pad mass in these leptin-treated GAL knockout mice, despite little change in food intake, indicates that this effect is primarily metabolic, reflecting perhaps the opposite effects of GAL and leptin on SNS activity [47]. The finding that GAL knockout mice respond normally to food deprivation confirms the proposal that GAL, in contrast to NPY and AgRP, is not a critical component in the metabolic response to starvation, at least in the short term.

In summary, GAL-expressing neurons in the PVN have very different properties and functions from other orexigenic peptides (Fig. 1). In contrast to NPY, AgRP and MCH, this peptide is unresponsive to food deprivation and to changes in leptin, CORT, glucose utilization and dietary carbohydrate. This indicates that GAL is not essential under conditions where food is scarce or on low-energy, high-carbohydrate diets. In contrast, GAL functions in close relation to dietary fat, and it mediates specific functions allowing animals to adapt to conditions of positive energy balance and excess fat. It is stimulated by a high-fat diet and an increase in circulating lipids, and it rises during the first half of the nocturnal feeding cycle as fat intake naturally rises. The function of this peptide on a high-fat diet is to restore carbohydrate balance, through behavioral and metabolic actions, under conditions where carbohydrate intake and metabolism are suppressed.

### 2.5. Orexins

The orexins, also referred to as hypocretins, are a recently identified class of neuropeptides [97,351]. Orexin A and orexin B, sharing 46% identity, are coded by the same gene and are localized in neurons of the PFLH. These orexin-expressing neurons lie adjacent to, but do not overlap, the cell population that produces MCH [50,115,315]. Orexin neurons project within the hypothalamus, including to the ARC and PVN, as well as throughout the brain [290]. Two orexin receptors, Ox-1 and Ox-2, have been described to date and display similar widespread expression patterns as orexin axons [159,415].

As with MCH and GAL, the orexin peptides in the PFLH are regulated differently in many respects from the orexigenic systems in the ARC [227]. Whereas they are similar to NPY/AgRP in being stimulated by food deprivation [58,277,351], glucocorticoids [393], and ghrelin [218,305,459], diametrically opposite changes in these peptides are detected in response to insulin, which stimulates orexin mRNA [133,279] while inhibiting NPY and AgRP, and also in genetically obese *ob/ob* mice, which exhibit a decrease in orexin mRNA [278,458] in contrast to an increase in NPY and AgRP. Further, orexin gene expression is reduced during pregnancy and lactation [126], states of increased energy demand that stimulate NPY, and their rhythm across the circadian cycle differs, with the orexins showing increased activity during the first half of the dark phase [265,463] rather than prior to dark onset for NPY. Also, whereas leptin receptors are found to exist on orexin neurons [143,167,447] and exogenous leptin reduces orexin

levels [32] and the activity of orexin neurons [459], there is little evidence for a direct interaction between leptin and the orexin peptides under physiological conditions. The orexins, in contrast to NPY and AgRP, remain stable or are actually increased in conditions or states with markedly elevated leptin, including overfeeding and dietary obesity [32,58,396,453,458]. Also, while NPY-induced feeding is completely inhibited by leptin or by anorectic peptides closely linked to leptin, the orexin feeding response is only partially suppressed by these compounds, suggesting the additional involvement of leptin-insensitive pathways [465].

Rather than leptin as a key regulator, there is accumulating evidence to suggest that orexin neurons in the PFLH are controlled by changes in circulating nutrients and diet that link short-term feeding behavior to long-term body weight control. They are stimulated when plasma glucose falls and food is withheld, and they are inhibited by glucose and signals related to the ingestion of food [48,57,58,133,459]. Building on evidence that neurons in the PFLH are “glucose sensitive,” i.e., stimulated by a decline in glucose [37], there are reports indicating that some of these neurons express orexin mRNA, which is stimulated by insulin-induced hypoglycemia [133,279] and by ghrelin which rises during hypoglycemia [413,414], and that orexin neurons may be the same as those containing “prolactinlike-immunoreactivity” which are similarly activated by hypoglycemia [333]. Based on evidence linking the orexins to arousal and the sleep–wake cycle, these findings have led to the proposal that this peptide system has a function in mediating hypoglycemic arousal induced by fasting [459].

In addition to being stimulated by a decline in glucose, orexin neurons are also found to respond, in the opposite direction, to circulating fat. This is suggested by a recent report showing orexin mRNA in the PFLH to be stimulated by the consumption of a high-fat diet as compared to a low-fat diet and to rise even further in association with obesity on a high-fat diet, which is characterized by hyperlipidemia [453]. This increase in orexin expression is positively related to levels of circulating triglycerides, leading to the proposal that the orexins respond similarly to GAL and that these peptides, and perhaps others, form a class of “fat-stimulated” peptides that are responsive to lipids in the blood [232,453]. Injection of Intralipid that raises triglyceride levels also stimulates orexin mRNA in the PFLH, similar to GAL in the PVN, and this effect occurs in the absence of changes in leptin, supporting a specific role for the circulating nutrients [453]. Consistent with this relationship of the orexins to circulating lipids is the circadian rhythm of orexin peptides, which like GAL show peak levels several hours after the onset of the natural feeding cycle [265,463], when rats exhibit a natural rise in their preference for dietary fat [374]. Taken together, these reports demonstrate that the orexin neurons are positively related to circulating lipids, while inversely related to glucose. They suggest a possible contribution of this peptide system to the increased activity as well as the acute hy-

perphagia associated with consumption of a high-fat diet [55,453].

Central injection studies demonstrate that orexin peptides, with orexin A more effective than orexin B, stimulate food intake, possibly through activation of both Ox-1 and Ox-2 receptors [351]. This effect, localized to the PFLH and PVN [394], is apparent only during the light period [153] and, as with MCH and GAL, is considerably smaller and of shorter duration than the eating response produced by NPY [109]. The finding that orexin A produces a stronger feeding response on a high-fat diet compared to a low-fat diet [82] provides further support for the idea that endogenous orexins contribute to the hyperphagia induced by a high-fat diet. The stimulatory effect of the orexins on locomotor activity, grooming and searching behaviors substantiates their proposed role in states of increased vigilance and arousal [139,171] that occur, for example, during periods of fasting and high-fat diet consumption. The orexin peptides additionally affect energy metabolism, as demonstrated by their stimulatory effect on metabolic rate independent of an increase in food intake or locomotor activity [253]. Further, they elevate sympathetic tone, as revealed by an increase in blood pressure and heart rate, autonomic efferent nerve activity and plasma catecholamines, and gastric acid secretion and luteinizing hormone release, effects generally seen at lower doses than those required to stimulate feeding [76,324,352,373]. The finding that orexins act on medullary neurons to increase sympathetic nerve activity raises the possibility that this peptide system underlies the increased sympathetic activation and risk of high blood pressure associated with obesity [345]. Thus, in addition to controlling feeding and energy metabolism, the orexins act at different levels of the neuroaxis, through extensive projections to the forebrain and spinal cord [95,96,127,290,315], to modulate hypothalamic regulatory systems involved in arousal and both neuroendocrine and autonomic processes. These physiological mechanisms may underlie the anti-obesity effects of an orally active orexin receptor antagonist [382].

Genetic studies support a role for the orexins in control of feeding behavior as well as activity level. Prepro-orexin knockout mice and mice harboring the toxic ataxin-3 gene attached to the orexin promoter, which eliminates orexin neurons, show hypophagia and inactivity compared to control littermates [147,447]. This inactivity may contribute to the unexpected, late-onset obesity exhibited by these mice [147,447]. Similar to mice and dogs with null mutations of the Ox type-2 receptor gene, these knockout mice show phenotypes similar to narcolepsy, a human sleep disorder [73,147,245,446]. Characterized by excessive sleepiness and abnormal sleep-wake regulation, narcolepsy is a slowly progressive disease associated with a gradual loss of orexin neurons in the PFLH with aging. Interestingly, human narcolepsy is accompanied by increased body mass index [166,294,357]. This evidence substantiates the idea that the obese phenotype of orexin knockout and orexin/ataxin-3 transgenic mice reflects an underlying decrease in energy

expenditure, due to reduced locomotor activity, foraging behaviors and metabolic rate. It is consistent with the results showing that orexin/ataxin-3 transgenic mice with ablated orexin neurons fail to exhibit the increased wakefulness and arousal induced by food deprivation [459]. Thus, rather than arising from greater hunger per se, the stimulatory effect of the orexins on food intake may be attributed, in part, to an increase in wakefulness and locomotor activity that is optimal for food-seeking behavior. This hypothesis is further strengthened by the finding that orexin A stimulates food intake during the light period, when animals are normally resting, but has no effect during the nocturnal feeding period [153].

Evidence suggests that the orexins, through their stimulatory effect on food-seeking behavior, act as downstream effector molecules of the NPY/AgRP systems, which reinforces signals related to hunger. In the hypothalamus, neuroanatomical studies suggest the existence of reciprocal synaptic contacts between orexin cells in the PFLH and neurons in the ARC that express NPY and AgRP [50,115,123,167]. Central injections studies show that orexin A stimulates Fos expression in NPY neurons [460] and, conversely, that NPY induces Fos expression in orexin neurons [292]. Consistent with this evidence are behavioral and pharmacological studies indicating the involvement of NPY receptors in mediating the feeding response induced by orexin peptides [69,175,460] and a role for the orexins, like NPY, in mediating the orexigenic effect of ghrelin [413], as well as the behavioral and metabolic responses to hypoglycemia as described above. In addition to NPY, the orexin neurons are anatomically linked to POMC neurons in the ARC [123,135]. Through this connection and their relationship to circulating lipids, the orexins may be involved in mediating the high-fat diet-induced increase in activity-based energy expenditure attributed to the melanocortins [55]. This suggests a possible function for the increased orexin expression observed on a fat-rich diet [453], in addition to the hyperphagia generally seen on this diet.

In conclusion, these results indicate that orexin neurons in the PFLH are highly responsive to changes in circulating and dietary nutrients (Fig. 1). They have similar properties to NPY/AgRP-expressing neurons in the ARC, in being stimulated by negative energy balance, such as after food deprivation, when glucose levels are declining and CORT and ghrelin levels are elevated. They are also similar to GAL-expressing neurons in the PVN, in being stimulated by dietary fat and circulating lipids and in exhibiting a natural rise during the first half of the circadian feeding cycle when fat ingestion normally increases. In both states of energy deficiency and of elevated fat consumption, the orexin peptides may provide a critical link between energy homeostasis and brain mechanisms coordinating sleep/wakefulness and motivated behaviors. They may function to increase arousal and locomotor activity that facilitates food-seeking behavior under conditions of fasting and in association with the

activity-based energy expenditure and hyperphagia induced by fat-rich diets.

### 3. Peptide systems that reduce feeding and body weight

#### 3.1. Central melanocortin system

Pro-opiomelanocortin (POMC) is a 267-amino acid precursor protein that is synthesized in the ARC as well as the anterior pituitary. The posttranslational processing of POMC results in a number of peptides with very different biological activities. In the anterior pituitary, it is processed predominantly to adrenocorticotropin-releasing hormone (ACTH) as well as to  $\beta$ -lipoprotein. In the ARC, ACTH is further processed to produce  $\alpha$ -MSH, whereas  $\beta$ -lipoprotein is cleaved to  $\beta$ -endorphin. Immunohistochemical studies show that  $\alpha$ -MSH terminals form a separate but parallel population to AgRP terminals [51]. The biological actions of the melanocortin peptides are mediated by interactions with at least five G-protein coupled receptors, with the MC3-R and MC4-R heavily expressed in the brain. Whereas the MC3-R is localized to the hypothalamus and limbic system and richly expressed in the ARC including on POMC neurons, the MC4-R is more widely distributed throughout the brain and is highly expressed within the hypothalamus, specifically in neurons of the PVN and PFLH [247,280,338]. Evidence described below indicates that the melanocortin peptides act through these receptors to exert a net catabolic action.

There is considerable evidence relating the melanocortins to circulating leptin and suggesting a role for this peptide system in mediating leptin's effects on energy balance. The expression of POMC mRNA in ARC neurons displays a diurnal rhythm, with nadir levels reached at the onset of the feeding cycle when leptin levels are low and peak levels at the end of the cycle [392]. The possibility that leptin stimulates these cells is supported by the findings that POMC neurons express the leptin receptor [79] and that this hormone rapidly induces c-Fos and SOCS-3 expression in POMC neurons [112], while stimulating POMC expression [359]. Leptin-deficient *ob/ob* mice exhibit a substantial reduction in POMC throughout the ARC, which is restored by leptin replacement [409]. Dietary obesity with elevated leptin is also accompanied by an increase in POMC mRNA [412,466], whereas states of food deprivation or lactation that have lower leptin levels are associated with reduced POMC gene expression [6,258,354,384]. Leptin stimulates  $\alpha$ -MSH secretion from hypothalamic slice preparations [194] and induces a concentration-dependent depolarization of POMC neurons [88]. This is possibly mediated by a non-specific cation channel, as well as a simultaneous reduction in the frequency of inhibitory currents from local NPY/GABA neurons [88]. Hypothalamic POMC neurons also express insulin receptors, and insulin is like leptin in stimulating POMC mRNA expression [35,152]. Similarly, POMC neurons express the

serotonergic receptor, 5-HT<sub>2C</sub>R, and agonists of this receptor as well as the 5-HT-releasing agent, D-fenfluramine, depolarize and induce fos-immunoreactivity in POMC neurons [157]. In contrast to the stimulatory actions of leptin, insulin and 5-HT agonists, the gut hormone, ghrelin, inhibits POMC neurons, possibly by stimulating NPY/GABA neurons that increase the frequency of inhibitory currents onto POMC neurons [89]. The relationship of glucocorticoids to POMC remains unclear, with some studies showing no change in POMC expression after ADX and others revealing a decrease after ADX that is reversed by steroid replacement [313,354,355,427,438].

Whereas few investigations of POMC have been conducted in relation to diet and nutrient metabolism, the available evidence indicates that the melanocortin system is relatively unresponsive to these signals. The expression of POMC in the ARC is unaffected by consumption of a high-fat diet [83,149,412]. Moreover, it is unaltered by compounds that block the metabolism of glucose or of lipids [366]. These findings distinguish POMC from the orexigenic systems described above and also from the CART peptide, which as described below colocalizes extensively with POMC [113] and is markedly stimulated by high-fat diet consumption [437,452].

Targeted POMC gene delivery into the basal hypothalamus of leptin-insensitive *fa/fa* rats, resulting in increased melanocortin signaling, causes a reduction in food intake and adiposity [242]. Consistent with these findings, central injections of  $\alpha$ -MSH or synthetic analogues with increased potency produce a robust suppression of food intake in obesity-prone and genetically obese mice [316,444,462]. AgRP knockout mice are more susceptible to the anorectic effects of the synthetic melanocortin peptide, MTH [378], supporting the concept that melanocortin peptides compete with AgRP at the MC4-R. These peptides also increase metabolic rate and sympathetic outflow [75,107], and they attenuate the cold-induced decline in body temperature in *ob/ob* mice [120]. Chronic administration of these peptides induces a transient decrease in food intake but a sustained reduction in body weight [180,316]. Further, continuous infusion of  $\alpha$ -MSH decreases intra-abdominal fat mass and improves insulin action on glucose uptake and production [297]. In contrast to the effects of  $\alpha$ -MSH, inhibition of MC4-R produces hyperphagia, increased fat mass, and hyperinsulinemia, and it ameliorates the catabolic actions of both leptin and insulin [132,297,303]. Thus, the actions of  $\alpha$ -MSH at the MC4-R favor enhanced SNS activity together with reduced food intake, which ultimately lead to a restriction of weight gain. There is recent evidence suggesting that  $\beta$ -MSH may also act at the MC4-R to control feeding [148].

Mice with a mutation in the coding region of the POMC-derived peptides, despite having defective adrenal development and low circulating glucocorticosteroids, develop hyperphagia and obesity, which are reversed by administration of  $\alpha$ -MSH [462]. A similar phenotype is seen in humans with a genetic POMC mutation resulting in a de-

iciency of  $\alpha$ -MSH [208]. In rodents, targeted disruption of the MC4-R gene, which diminishes the anorectic effects of the melanocortin agonist MTII [261], similarly causes hyperphagia, increased fat mass, and hyperinsulinemia [170]. A developmental study suggests that the magnitude of this response is attenuated by a compensatory increase in POMC mRNA and a decrease in NPY expression in these MC4-R knockout mice [439]. Spontaneous MC4-R mutations in humans are also linked to morbid obesity [162,421]. As revealed by a study in MC4-R-null mutant mice, this receptor in addition to decreasing food intake may have a function, independent of leptin, in mediating high-fat diet-induced thermogenesis and increased physical activity [55]. A similar role for the MC3-R in energy balance is suggested by the findings that mice lacking this receptor have increased body fat and that mice lacking both MC3-R and MC4-R weigh more than mice lacking only the MC4-R [54,74]. Similar to MC4-R-deficient mice, MC3-R-deficient mice also show indications of reduced activity and impairment in their ability to increase lipid utilization on a high-fat diet [54]. However, in contrast to the MC4-R knockout mice, MC3-R knockout mice are hypophagic and display a normal anorectic response to MTII [74]. Thus, whereas both receptors mediate pathways regulating energy expenditure and adiposity, only the MC4-R receptor appears to mediate the anorectic effect of the melanocortins. The profound obesity induced by these genetic mutations indicates that POMC-derived peptides, acting on central melanocortin receptors, mediate a tonic effect on the SNS under basal, freely-feeding conditions. This contrasts with the AgRP-deficient mice, which as described above exhibit a phenotype only in response to periods of food deprivation.

There is some evidence that the melanocortin system has a role in mediating disease-associated cachexia. Stimulation of MC4-R by melanocortin peptides reproduces some important features of cachexia, namely, a decrease in food intake and body weight and increase in metabolic rate. These effects induced by lipopolysaccharide, which stimulates cytokine release, are lost in MC4-R knockout mice [260], which are also resistant to cancer-associated cachexia [260]. This is in contrast to MC3-R knockout mice, which are actually more susceptible to the cachexia in disease models [259]. Since the MC3-R on POMC neurons is thought to act as an inhibitory autoreceptor [178], some of the features of these MC3-R mice may be mediated by facilitated  $\alpha$ -MSH release.

The melanocortin system appears to be closely related to the NPY neurocircuit. The POMC and NPY neurons are concentrated in the ARC, projecting to similar nuclei in the hypothalamus [51], and NPY axons project onto ARC POMC cells and inhibit their neuronal activity through the release of GABA [88]. Moreover, as described above, the melanocortin antagonist, AgRP, colocalizes with NPY and, similar to NPY, stimulates food intake and promotes body fat accrual [380]. Pharmacological studies demonstrate a complete suppression of NPY's orexigenic effect with in-

jection of melanocortin agonists [450]. Further, measurements of endogenous NPY and AgRP expression reveal opposite patterns to those detected with POMC. For example, POMC gene expression is increased in rats with dietary obesity [412], while NPY is generally reduced. Also, the circadian rhythm of POMC, with peak expression at the end of the dark cycle [392], is diametrically opposite to that of NPY mRNA, which peaks towards the end of the light cycle. This evidence supports the idea that the melanocortin and NPY systems interact antagonistically in the control of energy balance, although their functional relationship may differ somewhat in lean versus obese animals, with an intact leptin-signaling system required for the effect of C75 on POMC but not on NPY/AgRP neurons [371].

Together, the evidence indicates that POMC gene expression is stimulated under conditions of positive energy balance (Fig. 1). These include excess caloric intake, elevated leptin and insulin levels, and reduced levels of ghrelin, but they do not include conditions of high-fat diet consumption. Under basal conditions, a primary role of the POMC gene product,  $\alpha$ -MSH, in the hypothalamus is to provide a positive, tonic input via central melanocortin receptors on the SNS. During negative energy balance, increased levels of AgRP compete with  $\alpha$ -MSH and reduce its actions at the MC4-R. Under conditions of positive energy balance, increased release of  $\alpha$ -MSH serves to attenuate food intake and to further increase SNS activity to promote metabolic effects that restrict weight gain during excess caloric intake. As described below, possible downstream targets of the melanocortin signaling pathway may include GALP, CART and CRF, which mediate specific aspects of the day-to-day regulation of energy balance.

### 3.2. Galanin-like peptide

A 60-amino acid peptide structurally related to galanin, referred to as galanin-like peptide (GALP), was recently isolated and cloned from porcine hypothalamus [301]. This peptide shares sequence homology with GAL, and it binds with high-affinity to GAL receptors. However, clear differences exist between these peptides. Whereas GAL acts via both receptor subtypes, GALR1 and GALR2, GALP appears to be selective for GALR2 [214]. In contrast to GAL, GALP is expressed almost exclusively in the ARC [214], and these GALP-synthesizing neurons project to the PVN but not the lateral hypothalamus [397]. Relatively few GALP-containing fibers are evident in the external zone of the median eminence, suggesting that GALP, unlike GAL, does not serve as a hypophysiotropic hormone.

Further differences between these peptides can be seen in their expression patterns under different conditions. Whereas GAL mRNA in the ARC is not altered by leptin administration or food deprivation as described above, GALP in the ARC is clearly a target for leptin. The leptin receptor is expressed in the majority of GALP neurons, which constitute a novel neuronal population that is distinct from other

leptin-receptor expressing neurons in this nucleus [397]. Also, GALP-synthesizing neurons are stimulated by intracerebroventricular infusion of leptin, and their expression in the ARC is markedly reduced in leptin-deficient *ob/ob* mice, as well as in *db/db* obese mice and Zucker obese rats with a dysfunctional leptin receptor [181,182,206,209]. Food deprivation, which lowers leptin levels, reduces the rapid entry of circulating GALP into the brain [189].

Studies with manipulations of diet or circulating nutrients and their metabolism have yet to be performed with GALP. Thus, it is not known whether GALP is similar to GAL in its responsiveness to the consumption of a high-fat diet or to changes in the levels or oxidation of circulating lipids. The only evidence available relative to glucose is that the deprivation-induced reduction in entry of GALP into the brain is reversed by administration of glucose [189].

Central injections reveal further differences between the effects of GALP and GAL. With equimolar doses, these peptides produce distinct patterns of cellular activation in the hypothalamus, as revealed by Fos expression [122,219]. Whereas GAL induces a significantly greater number of Fos-positive nuclei in the PVN than GALP, there is a greater effect of GALP in the ARC, median eminence and other areas surrounding the ventricles. This differential neuronal activation may underlie the different behavioral and physiological effects of these peptides. While central GALP injection similar to GAL has an acute stimulatory effect on feeding in rats [206,217,267,367], this effect is not seen in mice [206]. Moreover, the long-term impact of this peptide in rats and mice appears to be a dose-dependent suppression of food intake and body weight [206]. Whereas the acute orexigenic effect of GALP may involve the GALR1 receptor as suggested for GAL [63,138,211,221,224], the receptor mediating GALP's feeding-inhibitory effect may be the GALR2, for which GALP has far greater agonist potency [214]. Alternatively, it may involve a novel GALP receptor. In addition to stimulating feeding, acute GALP treatment produces an increase in body temperature [217].

These central injection studies, revealing GALP's feeding-inhibitory and hyperthermic effects and its responsiveness to leptin, have led to the proposal that GALP is a downstream effector of leptin's actions in the hypothalamus possibly involving the melanocortins. This idea is supported by evidence that GALP coexpresses with POMC in some neurons of the ARC [398] and that leptin stimulates the release of GALP from hypothalamic explants [368]. Moreover, central GALP administration in leptin-deficient *ob/ob* mice decreases food intake and body weight, with the change in body weight being of longer duration, and it increases core body temperature and UCP1 mRNA in brown adipose tissue [146]. These effects suggest that leptin's activation of the SNS, resulting in increased energy expenditure and thermogenesis, may be partially mediated by GALP. It should be noted, however, that not all GALP neurons in the ARC express the leptin receptor [397]. Thus, there may be effects involving this subpopulation of leptin-insensitive

GALP neurons that differ from those produced by leptin and the melanocortins. These may include the acute stimulation of food intake and suppression of thyroid-stimulating hormone, effects similarly produced by GAL, and a stimulation of NPY release, an effect not seen with GAL [367]. Further evidence, indicating that GALP may function independently of the melanocortin systems and under different conditions, is provided by the finding that food deprivation, which suppresses POMC mRNA [38], has relatively little impact on GALP expression [209]. Preliminary evidence, showing GALP-containing axon terminals in direct contact with orexin- and MCH-containing neurons in the PFLH [399], suggests that this peptide may act, in part, by modulating orexigenic systems.

Taken together, this evidence indicates that GALP-expressing neurons in the ARC may be another feeding-inhibitory system that, like the melanocortins, is stimulated by states of positive energy balance and a rise in leptin (Fig. 1). This peptide produces catabolic effects, including a reduction in food intake and stimulation of thermogenesis, which help limit weight gain. However, GALP can also function independently of the melanocortins and generate different responses. This peptide, for example, has an acute, feeding-stimulatory effect and suppresses thyroid-stimulating hormone, similar to effects produced by its structurally-related peptide, GAL.

### 3.3. Cocaine- and amphetamine-regulated transcript

Cocaine- and amphetamine-regulated transcript (CART) was originally identified by differential display polymerase chain reaction as a novel mRNA stimulated after acute administration of psychomotor stimulants [103]. The predicted protein product of CART mRNA has a leader sequence and several pairs of basic amino acids, suggesting that it is processed and secreted. The mature peptide contains several cleavage sites, indicating that CART may be posttranscriptionally processed into long and short fragments, with both forms of CART peptide found in the rat and only the short-form peptide found in humans [102,103]. Whereas dense expression of CART mRNA exists in multiple areas outside the hypothalamus [169], the most abundant expression of this peptide is found in the hypothalamus [169], suggesting a role for this peptide in energy balance. Immunohistochemical evidence shows CART peptides in various nuclei of the hypothalamus, including the ARC, PVN and PFLH [203]. Further, CART-immunoreactivity is found in the pituitary and adrenal gland [203], suggesting a role in the hypothalamo-pituitary-adrenal (HPA) axis function, and also in sympathetic ganglia [106], indicative of a role in the sympathoadrenal axis. To date, no receptor for CART has been identified.

Investigations of the endogenous peptide show that CART expression is regulated by leptin [207]. It is stimulated by leptin administration or by hyperleptinemia induced by adenovirus-leptin treatment, and it is reduced in genetically obese *fa/fa* rats and leptin-deficient *ob/ob* mice with im-

paired leptin signaling [5,207,437]. It is also suppressed in the ARC in states of food deprivation, that decrease levels of leptin [6,336], and it is enhanced by chronic exposure to cold [201]. There is evidence that leptin administration activates CART-expressing neurons in the ARC, which innervate sympathetic preganglionic neurons in the thoracic spinal cord and possibly carry out the functions of leptin involving SNS activation [113]. Hypothalamic CART is also modulated by insulin and glucocorticoid status. It is reduced in streptozotocin-induced diabetic rats [243] and also in ADX animals, an effect reversed by dexamethasone administration [354,427]. Thus, CART responds in a positive fashion to insulin and the glucocorticoids, in addition to leptin.

Additional evidence shows that CART also changes in response to the consumption of fat and an increase in circulating lipids. The expression of CART in the ARC and PVN is stimulated in rats on a high-fat diet compared to a low-fat diet and also in rats that become obese compared to those that remain lean on a high-fat diet [437,452]. These changes may be attributed, in part, to the rise in leptin on a high-fat diet, with levels of this hormone positively correlated with the level of CART mRNA [452]. However, preliminary evidence from this lab demonstrates that CART can be stimulated by administration of Intralipid, which produces a rise in triglyceride levels without a change in leptin. Thus, whereas these results confirm a positive association between leptin and CART under physiological conditions, they demonstrate that CART is additionally responsive to circulating lipids, which also rise with body fat accrual on a high-fat diet.

Central injection studies support a role for CART in energy balance. Acute intracerebroventricular administration of CART peptide dose-dependently decreases food intake in rodents and reduces feeding stimulated by NPY [207,213,425]. Consistent with a tonic inhibitory effect of endogenous CART on feeding is evidence showing that chronic ventricular administration of this peptide lowers food intake and causes weight loss in lean and obese *fa/fa* rats [215] and that antibodies against CART cause an increase in nocturnal feeding [207]. It should be noted, however, that intraventricular administration of CART peptides can disturb locomotor activity [2,215] and, under certain conditions, induce a conditioned taste aversion [7], both of which may cause a suppression of food intake. Thus, the possibility of a stimulatory effect of hypothalamic CART on feeding needs to be considered [2]. This phenomenon has been substantiated by a follow-up study, which reveals a significant increase in daytime feeding in response to daily injections of CART into the ARC [201]. Other effects induced by central CART administration include an inhibition of gastric acid secretion and gastric emptying [302], increased levels of circulating free fatty acids [452], and stimulation of UCP1, 2 and 3 in brown and white adipose tissue and muscle, respectively [201,429]. These findings suggest that CART, like leptin, may have a role in stimulating thermogenesis. Based on evidence that CART is stimu-

lated by consumption of a high-fat diet [452], this peptide may mediate the thermogenic effect produced by fat consumption. It may also have a role in the thermogenic effect of cold stress, which elevates levels of CORT [4,402] that, in turn, may stimulate the expression of CART [354,427].

Studies in genetically modified rodents provide further support for a role of CART in thermoregulation. Using stereotaxically targeted gene transfer, rats receiving a CART transgene in the ARC compared to control rats exhibit an increase in food intake and a rise in UCP1 mRNA in brown adipose tissue [201]. This overexpression of CART is thought to mimic a state of chronic cold exposure, leading to a compensatory increase in food intake and consequent weight gain [201]. Whereas CART-deficient mice have normal body weight when maintained on a standard chow diet, they exhibit an increase in food intake, weight gain and fat mass when challenged with a high-fat diet at weaning [17]. These findings suggest that CART may play an important role in mediating thermoregulation, a process tightly coupled to food intake, and that the lack of CART impairs dietary-induced thermogenesis, resulting in greater body fat accrual on a fat-rich diet. The evidence that CART is stimulated by a high-fat diet and rises further with obesity on a high-fat diet [452] underscores the importance of this peptide's thermogenic effect in limiting body fat accrual under conditions of excess dietary fat. This peptide may act through activation of pathways innervating sympathetic preganglionic neurons in the thoracic spinal cord [113].

There is evidence supporting a relationship of CART to other hypothalamic peptides involved in energy homeostasis, with which CART coexists. In the ARC, virtually all CART neurons express POMC [113], in addition to the long-form leptin receptor [114]. This supports a close relationship of CART to the melanocortin system in stimulating thermogenesis and energy expenditure and in mediating the metabolic and feeding-inhibitory actions of leptin. In light of evidence that CART but not POMC is stimulated by a fat-rich diet, this specific function of CART itself may be exhibited only under certain conditions involving diets with a high-fat content. This idea is supported by evidence for a high degree of co-expression in PVN neurons of CART with the orexigenic peptide, GAL, which is stimulated by dietary fat [69], and by the finding that ventricular injection of CART reduces GAL production in the PVN while having no effect on NPY or AgRP, which are unresponsive to dietary fat [248]. Other studies reveal colocalization of CART with MCH in the PFLH [49], in addition to thyrotropin-releasing hormone in the PVN [49].

Together, the evidence demonstrates that CART neurons, particularly in the ARC, respond to a variety of conditions (Fig. 1). As with the melanocortins, they are stimulated under conditions of positive energy balance, in association with a rise in leptin, and they function to restrict food intake and activate the SNS, thereby preventing excess body fat accrual. The CART neurons, however, differ from the melanocortins in other respects. They fail to exert tonic effects under basal

conditions and, instead, are stimulated by short-term signals related to dietary fat and a rise in circulating lipids. They also respond to conditions of negative energy balance, such as cold exposure, possibly in relation to circulating CORT. The function of CART under these latter conditions may be to stimulate the SNS and increase thermogenesis to dissipate excess calories on a high-fat diet or maintain body temperature during periods of cold exposure.

### 3.4. Corticotropin-releasing factor

The corticotropin-releasing factor system in mammals comprises CRF, at least two different CRF receptor subtypes, a CRF-binding protein (CRF-BP) and endogenous CRF receptor ligands such as the urocortins [332]. CRF, a 41-amino acid peptide, is widely expressed in the brain, and CRF-synthesizing neurons are abundant in the medial parvocellular division of the PVN, where they control the pituitary–adrenal axis, exerting powerful regulatory effects on the release of ACTH and glucocorticoids [331,442]. Urocortins belong to the CRF family of peptides and are homologous to CRF, but they show a different pattern of distribution in the brain [161,332]. In the hypothalamus, urocortin I, a 40-amino acid peptide, is expressed primarily in the lateral hypothalamus and supraoptic nucleus, with urocortin-containing neurons projecting to the VMH [331]. Urocortins II and III are more recently identified members of the family [240,330]. In the mouse brain, urocortin II is expressed in the ARC and the magnocellular division of the PVN, whereas urocortin III expression is found in the rostral perifornical area lateral to the PVN [240,241,330,332]. CRF and urocortin peptides function through two G-protein coupled receptors, CRF<sub>1</sub> and CRF<sub>2</sub> [331]. CRF and urocortin I bind with high affinity to the CRF<sub>1</sub> [332,422]. In contrast, urocortins II and III bind with much higher affinity than CRF to CRF<sub>2</sub> and are suggested to be the endogenous ligands of this receptor [241,332]. In addition to binding to two receptors, CRF and urocortins also bind to CRF-BP, which is expressed in association with CRF-expressing cell groups in many brain areas including the hypothalamus [319] and has important modulating actions on CRF and urocortins [34,332].

The expression of CRF in the PVN is controlled by circulating CORT. Across the diurnal cycle, CRF mRNA and peptide levels exhibit a nadir at dark onset when CORT levels rise, and a peak at the end of the feeding cycle as CORT levels decline [56,276]. Also, CRF expression is increased in states of glucocorticoid insufficiency, such as after ADX, and this effect is reversed by glucocorticoid replacement [87,424]. This ADX state is associated with a decline in eating and body weight and impaired recovery of weight loss after food deprivation, and it prevents the development of essentially all forms of obesity [47,129]. This supports a permissive role for the glucocorticoids, perhaps in conjunction with insulin, in promoting body fat accrual [163,274]. In diabetic rats, CRF expression in the PVN is increased, perhaps

related to the activation of the HPA axis, and this effect is generally unaffected or further enhanced by the administration of insulin [67,68,335,377]. Physiological studies show that CRF is also stimulated in states of positive energy balance, such as involuntary overfeeding [361]. However, it is reduced in states of negative energy balance, such as food deprivation [118], and also in states of increased energy demand, such as during pregnancy or lactation [101,428], exercise [411], and cold exposure [151]. In light of its close relation to the diurnal cycle and its rise toward the end of the active phase, these expression studies suggest that PVN CRF may exert its inhibitory effect on feeding primarily during the later hours of the feeding period.

There are a few studies relating PVN CRF to diet and circulating nutrients. This peptide system, similar to POMC but different from CART, shows little change in response to excess fat consumption [72,272,356]. It is highly responsive, however, to changes in glucose levels or its utilization. This is revealed in an *in vitro* study, which shows a rise in peptide concentrations as glucose levels drop and a decline in CRF as glucose levels rise, an effect reversed by 2-DG [443]. Increased CRF expression in the PVN *in vivo* is seen in response to both insulin-induced hypoglycemia and 2-DG administration [67,309,334]. Thus, with a drop in glucose, CRF production may be increased to stimulate the release of glucocorticoids, whose major function is to maintain glucose levels within narrow limits. The importance of this relationship in maintaining normal eating patterns is suggested by the finding that sucrose drinking restores the disturbances in food intake, weight gain and sympathetic neural activity induced by ADX [216].

Central administration of CRF produces changes in food intake and metabolism that favor a state of negative energy balance [14,111,129,173,331,343]. The specific effects of acute CRF injections into the medial hypothalamus or PVN include a marked suppression of spontaneous or fasting-induced feeding. This is coupled with stimulation of sympathetic outflow and resting oxygen consumption, which increases lipolysis and energy expenditure and raises blood glucose while inhibiting insulin secretion. Central urocortin I administration also suppresses feeding, and this effect is strongest in the PVN and more potent and longer-lasting than that of CRF [94,205,385,430]. Also, chronic central administration of either CRF or urocortin I causes a sustained reduction in caloric intake and body weight [92]. The metabolic effects of these two peptides appear to differ. Whereas both CRF and urocortin I stimulate energy expenditure and urocortin I increases UCP1 mRNA in brown adipose tissue [202,332], chronic CRF but not urocortin I increases brown adipose tissue mass and raises circulating levels of CORT and lipids while reducing glucose [92]. Additional evidence suggests that CRF<sub>2</sub> mediates the anorectic effects of these ligands, while CRF<sub>1</sub> mediates their metabolic effects. This is supported by the findings that both urocortin I and CRF-induced anorexia are attenuated by an antisense oligonucleotide to CRF<sub>2</sub> but

not to CRF<sub>1</sub> [379] and that mice lacking the CRF<sub>2</sub> receptor show a blunted response to the feeding-inhibitory effects of urocortin [86]. Also, selective antagonists of the CRF<sub>2</sub> receptor block the suppressive effects of urocortins and CRF on food intake and body weight [92,332]. A role for endogenous CRF or urocortin I in energy balance is further supported by evidence showing an increase in feeding after pharmacological blockade of CRF receptors, expression or synthesis [156,173,331] and a reduction in food intake and body weight with chronic administration of CRF or a CRF-BP inhibitor that increases CRF/urocortin availability in the hypothalamus [155].

In addition to controlling basal and stress-induced activation of the HPA axis, CRF system has been implicated in mediating the integrated physiological and behavioral responses of stress, including a suppression of feeding, which are largely independent of the adrenal system and involve a direct action on brain receptors. This is supported, for example, by evidence showing that central blockade of CRF can reverse stress-induced anorexia [204,419]. The effects of CRF on metabolism and energy balance, as well as gastric emptying, may also involve alterations in immune signals, particularly cytokines [172,343]. This is suggested by the similarity of their effects and by the stimulatory effect of cytokines on CRF release and HPA axis. It is further supported by evidence showing the essential role of CRF in the hypophagic effect of interleukin-1 and its impact on fever, thermogenesis and ACTH release. This communication between CRF and the cytokines may be bi-directional, as suggested by the effects of CRF on immune and inflammatory responses [20,328]. Thus, the role of this peptide in energy balance must be evaluated in a broader context, as an integrator of the physiological responses to stress in relation to immunity and infection.

Genetic studies with CRF knockout mice show no disturbances in basal or stress-induced inhibition of food intake [281,395] or in their anorectic responses to lipopolysaccharide, interleukin-1 $\beta$  or D-fenfluramine [395]. However, CRF-BP-deficient mice show a decrease in weight gain together with reduced food intake [188], and, conversely, transgenic mice with widespread expression of CRF-BP exhibit increased weight gain, especially in female mice [250]. Further, in CRF<sub>1</sub> knockout mice similar to transgenic mice over-expressing CRF-BP in the anterior pituitary, the circadian rhythm of food intake is disturbed compared to wildtype mice, with more food ingested during the light and less during the dark period [53,282]. This effect in the CRF<sub>1</sub> knockout mice is completely reversed by CORT replacement, suggesting a potentially important role of CRF acting through the CRF<sub>1</sub> receptors in signaling a circadian rhythm of feeding behavior. Studies of CRF<sub>1</sub> or CRF<sub>2</sub> knockout mice have shed light on some of the anorectic characteristics of urocortin I. Whereas CRF<sub>1</sub> knockout mice are unresponsive to the acute anorectic effect of central urocortin I during the initial 1.5 h, they exhibit normal anorexia after 3 h and up to 11 h post-injection compared

to vehicle controls [44]. Conversely, CRF<sub>2</sub> knockout mice, which show urocortin I-induced anorexia during the first 4 h after administration, fail to exhibit any further anorectic response at 6 h and have normal cumulative food intake at 10 h post-injection [86]. Thus, the feeding-suppressive effect of urocortin I may actually have two phases, with an early phase mediated by the CRF<sub>1</sub> receptor and the long-term phase mediated by the CRF<sub>2</sub> receptor.

This role of CRF in inducing anorexia and weight loss may involve the NPY, melanocortin and CART systems, acting in a downstream fashion. The CRF neurons in the hypothalamus colocalize both the NPY Y5 receptor [59] and the MC4-R [251] and, thus, may provide an anatomical basis for communication between the NPY and melanocortin systems in the ARC and PVN. Consistent with this idea, CRF mRNA expression in the PVN is stimulated by central administration of a melanocortin agonist, in association with increased levels of CORT, and this effect is inhibited by an MC4-R antagonist [251]. Also, an antagonistic relationship between CRF and NPY is demonstrated by a variety of findings. This includes evidence that CRF and urocortin I administration reduces both NPY-elicited feeding and NPY expression [29,430], that ADX which enhances CRF also suppresses NPY mRNA, an effect reversed by glucocorticoids [12,62,144], and that CRF antagonists increase NPY feeding [156,271]. These studies support the idea that CRF acts, in part, by inhibiting endogenous NPY and that a reduction in CRF mediates the stimulatory effect of glucocorticoids on NPY. A further relationship of CRF to CART is suggested by evidence that central administration of CART peptide stimulates c-Fos immunoreactivity in CRF cells of the PVN [426], indicating that CRF may mediate the anorectic effect of CART. Consistent with these associations between CRF and the ARC systems responsive to leptin is a variety of evidence indicating a role for CRF in mediating leptin's actions. In addition to their similar behavioral and metabolic effects, the anorectic effect of leptin is attenuated by CRF antagonists, and central leptin injection increases CRF mRNA and peptide content as well as CRF<sub>2</sub> gene expression [266,295,331,358,420]. Leptin also facilitates the passage of peripheral urocortin I into the brain, potentiating its anorectic action [190]. A role for leptin in the relationship between CRF and NPY is supported by evidence for a negative feedback loop between leptin and glucocorticoids [62]. Studies also suggest an interplay between CRF and serotonergic pathways, showing that serotonergic agonists stimulate Fos expression in CRF neurons [43,176] and that CRF blockade attenuates the thermogenic effects of serotonergic agents [43,220].

In summary, the CRF neurons in the PVN are well known for being controlled by circulating CORT and downregulated at the start of the active phase as CORT levels rise (Fig. 1). This circadian rhythm helps to regulate food intake and activity level across the day–night cycle, which is determined by peptides like NPY and AgRP that increase at the onset of the natural feeding cycle. However, CRF also has

catabolic effects that involve restraining caloric intake and activating the SNS. Whereas CRF unlike the melanocortins does not appear to exert a critical tonic, inhibitory effect on sympathetic activity, it does respond to states of positive energy balance and, thus, may function downstream of the melanocortins, as well as the CART peptides.

#### 4. Conclusion

The study of eating behavior and obesity from the perspective of the brain is clearly a burgeoning field. There are many exciting discoveries, including the revelation of peptides for eating and satiety that were hitherto unknown. Technological advances have allowed investigators to define the specific functions of the different peptides in energy homeostasis (Fig. 1). It is clear that they exhibit considerable diversity as well as redundancy in their actions and are involved not only in the stimulation or inhibition of food intake but also in more intricate behavioral and physiological processes. These include, for example, the circadian rhythm of meals, the arousal of food-seeking behavior, and the regulation of energy expenditure as well as nutrient metabolism in different tissues.

The orexigenic peptides, NPY and AgRP in the ARC, function predominantly during states of fuel shortage, specifically reduced carbohydrate stores, and increased metabolic demand, and their goal is to restore carbohydrate balance as well as promote fat synthesis. They sense these states through hormones, namely, an increase in CORT and ghrelin and a decrease in leptin and insulin. They also respond positively to changes in circulating nutrients and their metabolism, specifically a decline in the levels, storage and utilization of glucose. In addition to a potent stimulatory effect on food intake, these peptides replenish carbohydrate stores and stimulate the utilization of excess glucose to produce anabolic effects including de novo lipogenesis. Whereas MCH in the PFLH also responds to negative energy balance, this peptide, unlike NPY and AgRP, can function independently of leptin and is unresponsive to ghrelin and nutrient-sensing signals indicative of reduced glucose availability and oxidation. With a relatively small effect on food consumption, its primary actions appear to be metabolic in nature, geared towards reducing activity level, energy expenditure, fat oxidation and thermogenesis. The GAL neurons in the PVN differ from the orexigenic peptides in the ARC, in being unaffected by negative energy balance and unrelated to changes in leptin, CORT, glucose utilization or dietary carbohydrate. Instead, GAL is highly responsive to dietary fat and circulating lipids. It has a specific function of stimulating carbohydrate metabolism in muscle, under conditions of excess dietary fat that compromise the utilization as well as intake of carbohydrate. Whereas the orexin neurons in the PFLH respond to negative energy balance similar to NPY/AgRP and MCH, they show clear differences in being highly sensitive to changes

in circulating nutrients, both glucose and lipids. The orexins sense negative energy balance predominantly through a decrease in circulating glucose, as well as an increase in CORT and ghrelin, and they act to stimulate feeding by increasing arousal and food-seeking behavior. They also sense positive energy balance induced by a high-fat diet and rise with circulating lipids, possibly contributing to the hyperphagia and activity-based energy expenditure associated with a fat-rich diet.

The anorectic peptides function predominantly during periods of fuel overflow, and their goals are to restrict excessive body fat accrual by limiting food intake and upregulating SNS activity. The POMC-derived peptide,  $\alpha$ -MSH, provides an important *tonic* input to these pathways, as reflected by the obesity exhibited by POMC-deficient mice. This distinguishes POMC from the other anorectic peptides, GALP, CART and CRF, which appear to have less critical inputs to energy balance under basal conditions. In states of positive energy balance, the actions of  $\alpha$ -MSH are increased further, and both CART and GALP in the ARC are recruited to help reduce feeding (although it should be noted that both peptides can also stimulate feeding) and activate the SNS to a level that restricts weight gain during excess caloric intake. The signals that trigger the recruitment of CART and GALP and increase POMC gene expression include elevated insulin and leptin levels and decreased levels of ghrelin, as well as increased dietary fat in the case of CART. Under negative energy balance, the actions of  $\alpha$ -MSH are inhibited by a rise in AgRP, which suppresses the activity of this peptide at the MC4-R. Another anorectic peptide CRF is also upregulated under conditions of positive energy balance. This peptide has a close association with circulating CORT and changes in glucose metabolism, and it plays an important role in mediating the circadian rhythm of food intake and activity. It may also act as a downstream mediator of  $\alpha$ -MSH and CART to exert catabolic effects, including feeding inhibition and SNS activation.

These findings illustrate how hypothalamic peptides do far more than just modulate food intake and have numerous other functions that contribute to energy balance, under different conditions and in different physiological states. It is clear that weight gain and body fat accrual can be increased through multiple mechanisms in addition to increased food consumption, including a stimulation of fat synthesis or carbohydrate utilization and a reduction in fat oxidation and energy expenditure. We recognize, therefore, that the terms 'orexigenic' and 'anorectic' are insufficient to describe the physiological functions of these peptides. This is further exemplified by the finding that both orexigenic (NPY/AgRP) and anorectic (CART) peptides in the ARC can function together, paradoxically, in unique situations such as cold exposure, with CART possibly acting more as a 'thermoregulatory' peptide that can enhance thermogenesis both in response to a high-fat diet and to cold. Genetic studies using knockout and transgenic mice have provided a valuable contribution to the validation of the physiologi-

cal functions of these peptides. Fasting studies in knockout models, for example, have demonstrated that peptides such as NPY and AgRP contribute to energy balance not by regulating basal food intake but by protecting the animal from perturbations in energy balance.

Collectively, the studies reviewed in this paper have advanced our understanding of the complex systems involved in energy homeostasis at the level of the hypothalamus. Such complex regulation is made possible by the large number of circulating hormones and metabolites that manipulate neuronal function and by the diverse anatomical and functional relationships that exist between the different peptides within the hypothalamus. It is also made possible by neural mechanisms, not reviewed here, that extend from the gut and other peripheral organs to the hindbrain and forebrain. A major miscalculation at any step within this complex circuit can lead to overeating and obesity. Whereas the unfolding beauty and intricacy of the neural and humoral systems involved is a thing of awe, its application in modern times is impossible without parallel appreciation of the enormous impact of the environment, in terms of food availability and diet composition. The complex systems of energy homeostasis evolved in an environment of scarcity. Thus, in a society where food is super-abundant and diets are rich in fat or carbohydrate, one needs socio-political help in coping with problems of body weight regulation. An understanding of the neuroscience of eating behavior and obesity, while critical to the discovery and development of future anti-obesity agents, must bring with it a sensible food distribution and education system. Otherwise, it will just be science for the sake of treatment, without all the benefits that could accrue from prevention of overeating.

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