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# Interacting Appetite-Regulating Pathways in the Hypothalamic Regulation of Body Weight*

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## I. Introduction

THE PAST decade has witnessed an upsurge in our understanding of the hypothalamic regulation of appetite. Expression of appetite or the motivational drive toward an energy source is a highly regulated phenomenon in vertebrates. It is considered a cornerstone for maintenance of energy homeostasis and for rigidly guarding the body weight around a set point. Abnormalities in the onset, periodicity, duration, and magnitude of eating episodes generally underlie augmented appetite (1–3). Increased appetite, whether temporary, as seen clinically in transient bingeing, or permanent, invariably culminates in an increased rate of body weight gain and obesity (1, 4–6). On the other hand, anorexia due to psychobiological causes (7, 8) or in response to acute and chronic infections, inflammation, and trauma is followed by severe loss of body weight (9–11). There is now a growing recognition that expression of appetite is chemically coded in the hypothalamus (1, 12). A perceived corollary is that subtle and progressive derangement in this neurochemical signaling, produced by environmental, genetic, and hormonal factors, impels either hyperphagia or anorexia (1). This conceptual advance has led not only to the identification and characterization of a multitude of neurotransmitters/neuromodulators that either propagate and transmit, or terminate appetite-stimulating impulses, but also to the precise tracking of pathways containing these signal molecules and the intricate interconnections among them. Increased knowledge of the molecular events governing synthesis, release, and signal transduction sequelae of each of these appetite-regulating messengers has enhanced our awareness of the phenomenon of coexistence and corelease of these chemicals in the cross-talk that ensues in response to an ever changing internal milieu (1).

Consequently, information amassed during this decade has revised our views on the hypothalamic control of appetite and helped to detail the mechanistic attributes of locally derived signals in regulating energy homeostasis. These attributes have crystallized into the following broad categories: 1) Embedded in the networks controlling a multitude of hypothalamic functions (1, 13, 14), there is a distinct circuitry regulating appetite. This circuitry is composed of an interconnected network of pathways elaborating and emitting orexigenic and anorexigenic signals (1, 15). 2) The neurons producing these orexigenic and anorexigenic signal molecules are subject to modulation by the internal milieu comprised of a variety of hormonal and other biologically active molecules. In this respect, the recent identification of the adipocyte protein, leptin, has renewed interest in feedback

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mechanisms between adipocytes and the appetite-regulating hypothalamic circuitry (16, 17). 3) A cascade of temporally related neural events in various components of the appetite-regulating network (ARN) precedes feeding episodes. 4) Emerging evidence supports the involvement of a distinct neural device for the timely onset of appetite expression, and disintegration of this control may result in unregulated food consumption. 5) A deficit in availability of orexigenic signal(s) at the signal transduction level, whether temporary or permanent, can perturb the postsynaptic receptor dynamics that eventuate in hyperphagia and increased body weight gain indistinguishable from that produced by excessive production and release of orexigenic signals (1, 18, 19). 6) Coexistence and corelease of orexigenic signals (20–22), along with the redundant overlapping and interconnected orexigenic and anorexigenic signaling pathways within the hypothalamus (1, 12, 15, 23), provide a microenvironment wherein subtle perturbations shift signaling in favor of unregulated hyperphagia rather than anorexia. The central theme of this article is to critically review our understanding of these fundamentals underlying neural control of appetite and to collate the growing information on several newly identified messenger molecules. Emphasis is placed on the anatomical distribution of signal-producing pathways in the hypothalamus, the site and mode of action of peripheral signals, and the cellular and subcellular events underlying hyperphagia and obesity in experimental and genetic models. In doing so we will present a conceptual model, which encompasses a broad spectrum of appetite-regulating messenger molecules, to explain the dynamics of the neural circuitry involved in stimulation and inhibition of appetite.

## II. Neuroanatomical Substrate for Appetite Regulation

Historically, a few discrete nuclei in the basal hypothalamus have been viewed as crucial in the regulation of daily energy homeostasis, especially those sites connected with neural mechanisms affecting appetite. The inference that hypothalamic sites, such as the ventromedial nucleus (VMN), dorsomedial nucleus (DMN), paraventricular nucleus (PVN), and lateral hypothalamus (LH), contain neural mechanisms affecting ingestive behavior was based on the results of numerous studies employing either discrete lesions in the hypothalamus or surgical transection of neural pathways (24–30). With the exception of lesions in the LH, these experimental manipulations within the brain disrupted the daily food intake pattern to produce permanently enhanced appetite or phagia. The inference from these investigations, that appetite-regulatory mechanisms may be confined to a few morphologically well defined regions in the hypothalamus, generated voluminous information on the patterns of ingestive behavior and the attendant shifts in endocrine and autonomic systems in rats undergoing these experimental manipulations (3, 13, 26, 30). Fundamental questions regarding the site(s) of propagation of appetite-stimulating impulses and food-seeking behavior, the transmission pathways and target sites in causation of appetitive behavior, however, remained unanswered for quite some time.

Recent intensive research, first for identification of orexigenic and anorexigenic neurotransmitters in the hypothalamus, followed by identification of the neuronal sites of their production, release, and receptive fields, has changed the landscape of the neuroanatomical substrate underlying ingestive behavior. Also, evidence of the morphological relationships among these neurotransmitter/neuromodulator-producing neurons, and the fact that these neurons can coproduce more than one appetite-regulating signal (1, 13, 20, 21, 31–41), have strengthened the concept, enunciated earlier (1, 12), that a distinct interconnected circuitry operates locally in the hypothalamus to regulate ingestive behavior. The following are the currently known anatomical components of this hypothalamic circuitry (Fig. 1).

### *A. Arcuate nucleus*

The arcuate nucleus (ARC) is located at the base of the hypothalamus on either side of the third cerebroventricle (Fig. 1). It extends rostrocaudally from the posterior borders of the optic chiasm to the mamillary bodies. This nucleus has recently gained prominence among the sites associated with the hypothalamic integration of energy balance due to two reasons. It contains a high density of neurons that produce the orexigenic peptides, NPY (21, 41), the opioids, dynorphin, and the POMC-derived peptide, β-endorphin [β-END (42–44)], galanin [GAL (45, 46)], and the amino acids, γ-aminobutyric acid [GABA (47, 48)] and glutamate (49, 50). Interestingly, αMSH, an anorexigenic peptide derived from the POMC precursor protein, is also coproduced with β-END in the ARC (51–53). The agouti-related transcript (ART) that encodes the anorectic agouti-related protein (AgrP), the selective antagonist of MC3-R and MC4-R receptor subtypes (54, 55), is coexpressed with NPY mRNA in the ARC (39, 40). The terminal fields of these orexigenic and anorexigenic producing neurons in the ARC extend into various hypothalamic sites including the VMN, DMN, perifornical hypothalamus (PFH), PVN, and preoptic area (POA) where microinjection of these neurotransmitters/neuromodulators affects feeding behavior (see respective sections dealing with these signal molecules).

Morphological evidence has demonstrated that a subpopulation of neurons in the ARC coexpress NPY and GABA (36–38), and NPY-producing neurons are synaptically linked with β-END- (34) and GAL-producing neurons in the ARC (33). Also, GAL-producing neurons establish morphological and functional links with β-END-producing neurons within the ARC (35). Further, experimental evidence has demonstrated that an interconnected orexigenic network is likely to operate in the hypothalamic control of the daily patterning of food intake in which synergistic action of NPY and GABA (37, 38) and regulation of β-END (34, 56) and GAL (35) release by NPY may play predominant roles. Recently, cocaine and amphetamine-regulated transcript (CART) mRNA has been localized in the ARC, and CART peptides appear to be physiologically relevant anorexigenic signals (57–61). In addition, due to the absence of blood brain barrier, the ARC is strategically positioned to be in direct communication with peripheral signals, such as the circulating adrenal and gonadal steroids as well as the large peptides, leptin and insulin, and

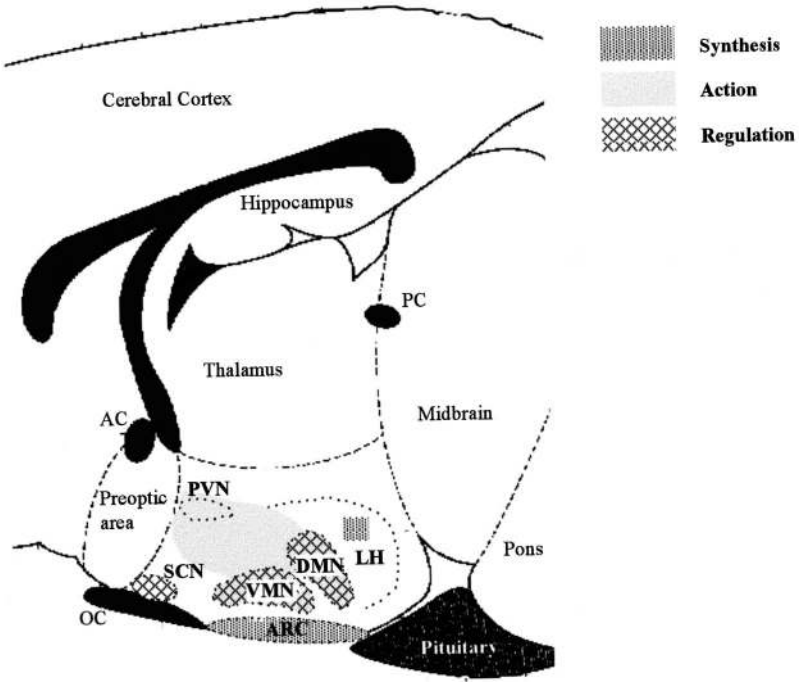


FIG. 1. A diagrammatic representation of hypothalamic sites associated with appetite-regulating signal pathways on a sagittal section near midline of the rat brain. AC, Anterior commissure; OC, optic chiasm; PC, posterior commissure. Synthesis, Hypothalamic sites involved in synthesis of orexigenic and anorexigenic signals. Action, Hypothalamic areas where orexigenic and anorexigenic messengers act. Regulation, Hypothalamic sites involved in regulation of synthesis, release, and action of orexigenic and anorexigenic signals.

the signals transported via the cerebrospinal fluid in the cerebroventricular system (14).

*B. Ventromedial nucleus and lateral hypothalamus*

Lesions in the ventromedial hypothalamus (VMH) that include the ventromedial nucleus (VMN) produce rapid hyperphagia and abnormal body weight gain that persist for a long time (24–26). The hypotheses that VMN is a "satiety center" exercising constant restraint on feeding and destruction of neuronal elements in the VMN permanently abolishes this restraint to allow sustained, unregulated phagia have been seriously contested over the years (13, 28–30). Evidence that parasagittal transections of fibers, between the LH, a hunger center, and the VMN extending up to the anterior hypothalamus, reproduced the VMH lesion-type hyperphagia and obesity challenged the role of the VMN in regulation of appetite (28, 30). However, in view of the emerging knowledge of the existence of an ARN extending over several hypothalamic sites, it is highly likely that the hyperphagia and obesity produced by VMH lesions and by transections of discrete pathways traversing the VMN are two distinct syndromes involving disruptions in disparate pathways (18, 19, 62). For example, microinjection of colchicine to disrupt neural signaling in the VMN diminished the availability and release of NPY in the ARC-PVN but augmented the galaninergic signaling in the hypothalamus (63–67). In addition, NPY gene expression was induced in hypothalamic sites that normally do not express NPY (68). Also, recent evidence clearly shows that VMH lesions disrupt signaling differently in each of the orexigenic pathways (see *Section III.A.1* and Refs. 63–68).

Although there is no evidence yet of production of either orexigenic or anorexigenic signals in the VMN, selective destruction of cell bodies in the VMN with ibotenic acid resulted in hyperphagia and increased rate of body weight gain (69) thus reemphasizing the role of neuronal elements in the VMN in regulation of appetite. On the other hand, microinjection into the VMN of NPY (13, 70), GAL (71–73), GABA, or GABA-agonist (74–77) and $\beta$-END (13, 74) stimulated feeding, while injections of leptin inhibited feeding (78, 79). Interestingly, microinjection of urocortin, a potent anorexigenic peptide, into the VMN also inhibited feeding (80). These findings raise the possibility of a receptive field in the VMN for several appetite-regulating signal molecules. The argument that spread of these signals through the extracellular fluid to receptive sites in the neighborhood most likely affected feeding is tenuous because receptors for each of these signals do exist in the VMN (see sections on each of these signals).

Further, the VMN is neurally linked with several hypothalamic sites implicated in the control of ingestive behavior. Although VMN efferents to the ARC have not been described, the VMN receives NPY (21), $\beta$-END (43, 44), and CART (59, 61) containing projections from the ARC. VMN efferents to the DMN and parvocellular subdivision of the PVN (pPVN) have been traced, thereby supporting the possibility that disruption in signaling within the VMN may perturb flow of information to the DMN-PVN axis for the release of orexigenic signals, the consequence of which is unregulated feeding (81–84).

LH is a contiguous band dorsal and lateral to VMH, extending rostrally from the mesencephalic tegmentum to the lateral preoptic area. In addition to sparsely distributed neuronal subpopulations, it is the site of passage for the medial forebrain bundle and other fibers connecting forebrain and midbrain structures with each other and several hypothalamic sites. Lesions in the LH produced temporary aphagia, adipsia, and loss in body weight (24–27, 85). The severity of the LH syndrome and near normal recovery of food intake and body weight depended upon the location and the size of

the lesion (85). These observations led Anand and Brobeck (24, 25) to conceptualize LH as a "feeding center" for elaboration of "urge to eat" normally restrained by signals from VMH. The fact that daily electrical stimulation of LH produced vigorous feeding leading to increase in body weight (86, 87) suggests the possibility that LH stimulation either restrained the action of an orexigenic signal(s) or activated those orexigenic pathways originating locally or in the vicinity of the LH. Indeed, recent evidence that melanin-concentrating hormone (MCH), orexins, and excitatory amino acid (EAA) glutamate are produced in the LH and microinjection of orexin and glutamate agonist stimulates feeding strengthen the view that LH is an integral part of the ARN in the forebrain (for details see *Sections III.D, III.E,* and *III.F*).

### *C. Dorsomedial nucleus*

Electrolytic lesions in the dorsomedial nucleus (DMH) disrupt feeding to a far less extent than lesions in the VMH (81). As in other hypothalamic sites, microinjection of various orexigenic signals in the DMH elicited feeding (13, 70–77). A crucial role of neurons in the DMN was indicated by the observation that inhibition of NPY-induced feeding by leptin enhanced neuronal c-FOS in the DMN, the protein product of the immediate early gene and a marker of neuronal activation (88–90). These findings were interpreted by us to infer that the site of NPY and leptin interaction may reside in the DMN and may represent a component of the circuitry involved in either attenuation or inhibition of feeding by leptin (89, 90).

DMN efferents have been traced to the VMN, and the two subdivisions of the PVN, the pPVN and magnocellular PVN (mPVN), with densest projections seen in the pPVN (81, 83, 84, 91, 92). Because there are prominent ARC efferents containing NPY to the DMN (93), it is highly likely that NPY released in this nucleus participates in stimulation of feeding. However, NPY levels in the DMN, unlike that seen in the PVN and ARC, were not elevated in response to fasting (94). Another relevant finding is that although only a few neurons in the DMN normally express NPY (95, 96), NPY gene expression was increased severalfold in the DMN in association with hyperphagia induced by disruption of neural signaling in the VMN (68) and in a genetic model of obesity and in response to lactation-induced hyperphagia (Refs. 97 and 98; see *Section III.A.4*). It is not clear whether augmented NPY gene expression is associated with increased NPY availability in DMN efferents to the PVN and surrounding sites in these models of hyperphagia.

### *D. Paraventricular nucleus and perifornical hypothalamus*

The PVN ranks second only to the VMH in terms of investigative interest in the hypothalamic control of appetite (Fig. 1). A dense cluster of heterogenous neurons fan out within a well defined boundary of the PVN on either side of the roof of the third cerebroventricle in the hypothalamus (91, 99). Several lines of evidence suggest that neuronal elements in the PVN participate in the control of ingestive behavior. Microinjection into the PVN of virtually all the known orexigenic signals, NPY, GAL, orexins, GABA, opioids, norepinephrine (NE), and epinephrine (E), stimulated feeding (13, 70–77, 100), thereby implying the existence within the PVN and its vicinity of receptor sites for each of these signals. Additionally, microinjection of the anorexigenic neuropeptides such as CRH, produced locally (101, 102), and leptin (78), attenuated fasting-induced feeding. Furthermore, activation of the early gene marker, c-FOS, was augmented in neurons in the PVN in response to administration of orexigenic (103, 104) and anorexigenic signal molecules (88–90, 105). Another noteworthy observation is that among other hypothalamic sites tested, such as the VMN, the PVN is the only hypothalamic nucleus in which release of NPY, the most potent orexigenic signal, was augmented both *in vivo* and *in vitro* in response to fasting and before initiation of feeding (106, 107). These seminal findings are consistent with the idea that the PVN is one of the crucial sites for the release of orexigenic signals, and also possibly one of the sites of interaction of neurotransmitters/neuromodulators that inhibit feeding by diminishing NPY release.

Whereas evidence from various experimental paradigms reinforces the notion that the PVN is a crucial site for action of orexigenic signals, it is intriguing to find that destruction of the PVN also evoked hyperphagia and abnormal body weight gain accompanied by modifications in endocrine and autonomic profiles that were quite different from those produced by VMH lesions (108–110). It is possible that, under these conditions, neural elements surrounding the PVN compensate for the PVN loss. The PFH, the neural tissue surrounding the fornix and rostral to the LH, was reported to be relatively more effective in stimulation of feeding than the PVN after the microinjection of orexigenic signals such as NE and NPY (13, 111, 112), and orexin A microinjection into the PFH stimulated feeding (100). However, involvement of the PFH within the orexigenic circuitry is rather tenuous because of the strong possibility of diffusion of NPY and NE from the PFH to the PVN and lack of evidence correlating altered secretion patterns of orexigenic signals in the PFH in response to shifts in energy balance, as seen in the PVN (106, 107).

### *E. Suprachiasmatic nucleus and the timing device*

The pattern of ingestive behavior is a highly regulated phenomenon in all living organisms. The drive to eat is evoked by appetite or the sensation of hunger which, in most vertebrates, is neurally based and entrained to activity-arousal mechanisms in the light-dark cycle. For example, rats consume between 85–90% of their total intake during the lights-off period. Ingestive behavior is initiated soon after onset of the dark phase, which apparently provides either the "trigger" or intensifies the drive for food (1). On the other hand, in nonhuman primates maintained in the laboratory and in humans, signaling of appetite is linked to socially acceptable or individually based requirements. Generally, neural, metabolic, and hormonal signals that influence ingestive behavior are stable during the period preceding and at the onset of the drive to eat (1, 113). The negative energy balance, which occurs during fasting, dieting, or undernourishment, intensifies the appetite to prevent underconsump-

tion, but it is unlikely by itself to be the timely "trigger" for the drive to eat.

In addition, experimental findings invoke the timing mechanism in generation of the sensation of hunger. Discrete lesions in the suprachiasmatic nucleus (SCN), two small round nuclei resting dorsally on the optic chiasm on either side of the third cerebroventricle (Fig. 1), result in loss of regulated feeding (114, 115). Destruction of the VMH or disruption of neural signaling in the VMN similarly results in unregulated phagia (18, 19, 26). In humans, several instances of abnormal patterns of feeding characterized by a loss of regulated consumption of meals and appearance of the nighttime binge syndrome are associated with hyperphagia and obesity (2, 116).

In the rat, the photoperiodic-dependent circadian pattern of feeding is undoubtedly linked to information from the SCN to the hypothalamic ARN in two ways (Fig. 1). It is likely that initiation of nighttime feeding results from the increased release of orexigenic signals (see *Section III.A*). This may occur either directly in the orexigenic network or indirectly by restraining the influence of anorexigenic messengers on the release and action of orexigenic signals (see *Section IV* and Fig. 7). Conversely, reinstatement of the anorexigenic influence in a timely manner during the lights-on period may enforce cessation of feeding. The evidence that lesions in the SCN resulted in uninterrupted feeding is in agreement with the notion that the SCN exerts a restraining influence on ingestive behavior through the release of anorexigenic signals. A circadian pattern in hypothalamic gene expression of the orexigenic signals, NPY, GAL, and β-END-generating precursor (POMC; Refs. 117–123), is also consistent with a regulated pattern of daily energy management. Interestingly, gene expression of these peptidergic signals peaked between 0700–1500 h during the lights-on period, followed by a trough before and during the dark phase when rats normally eat (Fig. 2 and Refs. 120, 121, and 123). Consequently, whereas this anticipatory augmentation in gene expression of orexigenic peptides may be needed for the supply of orexigenic peptides during the dark phase, undoubtedly feeding is regulated independently by circadian impulses that evoke the release of these peptides. Thus, we propose the existence of distinct neural mechanisms, one for synthesis and the other for release of orexigenic signals, emanating from the SCN and possibly other neural timing devices in the brain.

It is evident that the SCN efferents shown to terminate in the VMN, DMN, subparaventricular zone, and LH constitute direct lines of communication to components of the orexi-

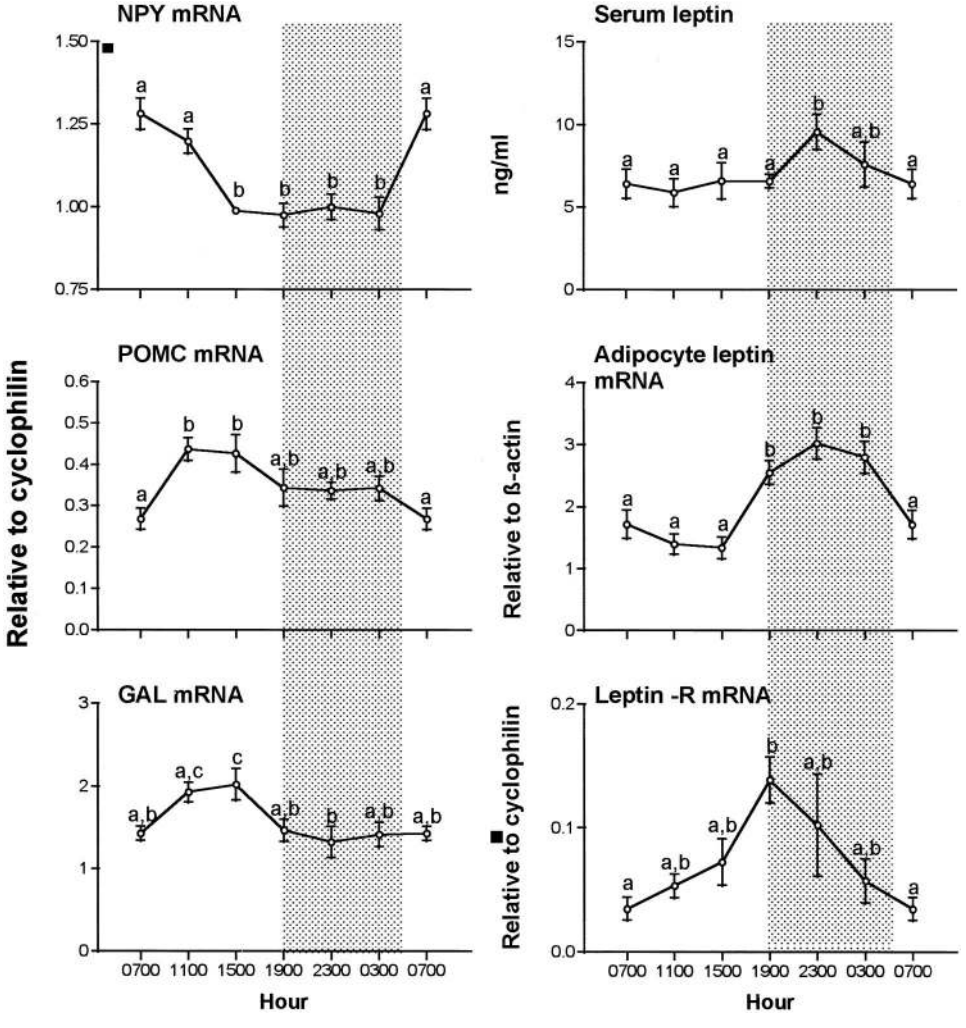


FIG. 2. Dynamic changes in hypothalamic NPY, POMC, and GAL mRNA (*left panel*) and serum leptin, leptin mRNA in adipocytes, and hypothalamic leptin receptor (R) mRNA levels (*right panel*) over a 24-h period. *Shaded columns* indicate the dark phase (1900–0500 h). *Dissimilar superscripts* are significantly different ($P < 0.05$).

genic network for causation of circadian ingestive behavior. These efferent pathways may employ vasoactive intestinal peptide, vasopressin, and GABA as neurotransmitters. The neurons in the SCN and ARC are linked because SCN efferents innervate NPY, GAL, and POMC producing neurons in the ARC. However, it is unknown whether the daily pattern of gene expression of these peptides in the ARC is regulated directly or via projections to the DMN and/or the VMN (Fig. 2 and Refs. 124–135).

### *F. Overview of the neuroanatomical substrate associated with appetite control*

Although it is now possible to identify the hypothalamic site or sites involved in regulation of appetite, the precise location of the receptive neural sites for each of the orexigenic and anorexigenic signals has not yet been ascertained (Fig. 1). The receptors for these signals are highly concentrated in the PVN, but they are by no means restricted to this site. The evidence that microinjection of orexigenic signals in a number of hypothalamic sites affects feeding argues strongly for a receptive field that extends beyond the PVN. The fact that lesions of the ARC, VMN, DMN, or PVN failed to subdue the drive to eat underscores a widespread receptor field associated with appetite regulation. This observation, coupled with the reports 1) that neural receptive sites for the anorexigenic signals, such as leptin, insulin, CRH, urocortin, and CART, overlap with sites containing receptors and terminal fields of orexigenic signals, including OR (see *Sections IV and V*) and 2) that orexigenic and anorexigenic neural systems are morphologically and functionally connected, strongly support our proposal of the existence of an interconnected ARN in the hypothalamus. We propose further that the ARN, spanning over several hypothalamic sites including the SCN, is composed of diverse signals (Ref. 1; Figs. 1 and 7). Disruptions, either permanent as produced by lesions or neurotoxins in any part of the ARN, or temporary as caused by imbalances in hormonal and autonomic afferent signals, are likely to derange the tight control in the daily management of energy homeostasis leading to hyperphagia, abnormal body weight gain, and obesity.

## III. Orexigenic Signals

### *A. Neuropeptide Y*

In the recent past, no other brain-signaling modality has drawn more attention than neuropeptide Y (NPY) in the control of appetite, body weight gain, and obesity. The potent appetite-stimulating effects of NPY and other members of the pancreatic polypeptide (PP) family were reported (136, 137) soon after Tatemoto (138) isolated and characterized the amino acid sequence of NPY, a member of the PP family. Subsequent research, spanning over a decade, has affirmed the hypothesis that NPY is a naturally occurring appetite transducer, and under normal conditions NPYergic transmissions represent an essential component of the common final pathway in the hypothalamic integration of energy homeostasis (1, 139). The wealth of information regarding NPYergic control of appetite is collated below.

#### *1. Neuroanatomical pathways.*

*a. Source of NPY:* Although NPY-producing neurons are located in several sites in the brain (21, 139, 140), two subpopulations, one representing the extrahypothalamic cluster in the brainstem (BS) including the locus coeruleus, and the other located in the hypothalamus along the length of the ARC and in the DMN (1, 139), apparently participate in a disparate manner in the daily management of food intake (1, 139, 141).

NPY-producing neurons in the BS innervate various hypothalamic sites including the ARC, VMN, DMN, PVN, and surrounding regions (21, 140–142). A distinguishing feature of this population is that three other orexigenic signals, the catecholamines, NE and E, and the peptide GAL, are coproduced with NPY (21, 22). Consequently, when coreleased, these orexigenic signals are likely to interact at hypothalamic target sites (143). Experimental evidence suggests an interplay between NPY and the adrenergic system (141, 143). Transection of BS projections to the hypothalamus showed that whereas practically all the NE and E in the hypothalamus originated in the BS, only 40–50% of the NPY in various hypothalamic sites was derived from the BS (141, 142). Elimination of these BS inputs into the hypothalamus, either surgically (141, 142) or by injection of the neurotoxin, 6-hydroxydopamine (6-OHDA; Ref. 62), resulted in hyperphagia, a progressive increase in body weight and obesity. Hyperphagia in these rats was characteristically seen during the dark phase (Ref. 62 and our unpublished results). The cellular and molecular events causing increased phagia and body weight gain in these NPY- and catecholamine-deficient rats have been elucidated recently (Ref. 62; see *Section III.A.4*). The findings are consistent with the view that NPY of extrahypothalamic source along with the coexisting orexigenic signals is engaged in genesis and consolidation of neural stimuli that initiate and regulate nocturnal feeding.

Since the early demonstration that rapid time-dependent changes in NPY levels occurred in various hypothalamic sites in rats in response to fasting and refeeding (94), much attention has been directed toward defining the role of the population of NPY-producing neurons in the hypothalamic ARC. Studies involving lesions in the ARC and retrograde tracing studies demonstrated that approximately 15–20% of ARC NPY neurons innervate the PVN and DMN (93, 144). These quantitative data, together with the reports that the BS neurons contribute 40–50% of NPY in the PVN and other hypothalamic sites (141, 142), imply that the remainder of NPY in hypothalamic sites is produced either locally in the PVN or in neurons located in the vicinity. The DMN may normally be a small source of NPY in the PVN and elsewhere in the hypothalamus (93, 142), but it may contribute heavily in response to challenges that demand increased phagia (68, 97). Similarly, little NPY mRNA is detected in the PVN of normal rats but in response to disruption of neural signaling in the VMN, there is up-regulation of NPY gene expression (68). The possibility that the VMN is also a likely source of NPY for various hypothalamic sites because lesions in the VMN reduced NPY levels in the PVN and other selected hypothalamic sites (145, 146) remains to be investigated.

*b. Where does NPY act to stimulate feeding?:* Since administration of NPY into the third cerebroventricle readily stimulated feeding in satiated rats, it was predicted that the site(s) of NPY action may reside in and around the paraventricular region of the hypothalamus (Fig. 1; Refs. 136 and 137). Indeed, direct application of minute amounts of NPY into various hypothalamic and extrahypothalamic sites stimulated feeding (13, 70), and the PFH, the region lying caudal to the PVN, was reported to be more responsive to NPY than other sites in the neighborhood, including rostral structures such as the POA (70, 112). Also, demonstrations that injection of NPY into the fourth cerebroventricle stimulated feeding raised the possibility that the NPY-receptive field may extend outside the hypothalamus, possibly into the BS (104). Thus, as proposed in the preceding section, it is highly likely that the field of NPY action in stimulation of appetite is widespread in the rat brain (Fig. 1). This is supported by the localization of NPY $Y_1$ (18, 19, 147–155) and $Y_5$ (156–161) receptor subtypes, putative receptors mediating stimulation of feeding by NPY, in sites corresponding to those where microinjection of NPY stimulated feeding (70).

Neuroactive substances, such as NPY, when microinjected into discrete neural sites or into the cerebroventricle, travel, through volume transmission modality, in the extracellular fluid to remote sites (162, 163). Our findings that NPY injection into either the third or the fourth ventricle stimulated c-FOS in similar forebrain areas (103, 104) argues for this volume transmission modality, *i.e.*, movement of signals in interstitial fluid (163), to transport NPY to distant NPY target sites. Even the endogenously released NPY in the PVN can travel to surrounding hypothalamic sites that contain NPY receptors and are responsive to NPY microinjection. A comparison of the topography of c-FOS activation by NPY in the brain revealed the PVN and DMN as sites closely associated with NPY-induced feeding (103, 104). Indeed, pretreatment of rats with either the NPY $Y_1$ receptor antagonist 1229U91 (164) or leptin (Fig. 3; Ref. 90) attenuated NPY-induced feeding and c-FOS activation in the mPVN, and leptin + NPY treatment enhanced c-FOS in the DMN. This line of investigation revealed that NPY targets engaged in stimulation of feeding reside within the DMN-mPVN axis (90, 164).

An additional site of action is apparently the ARC itself, where NPY-producing perikarya abound and where NPY immunoreactive fibers from the BS terminate (21) and NPY collaterals are abundant (165). Intracerebroventricular injections of NPY into the third or fourth cerebroventricle increased the number of c-FOS-immunopositive neurons in the ARC (103, 104), possibly by activating $Y_1$ and/or $Y_5$ receptor subtypes located on peptidergic neurons (148, 149, 156, 157, 166, 167). Recently Rhim *et al.* (168) reported that NPY elicited a long-lasting synaptic inhibition in the ARC through inhibition of GABAergic and glutaminergic transmission. NPY-producing neurons also synapse in the ARC with neurons producing orexigenic signals such as POMC and GAL (33,

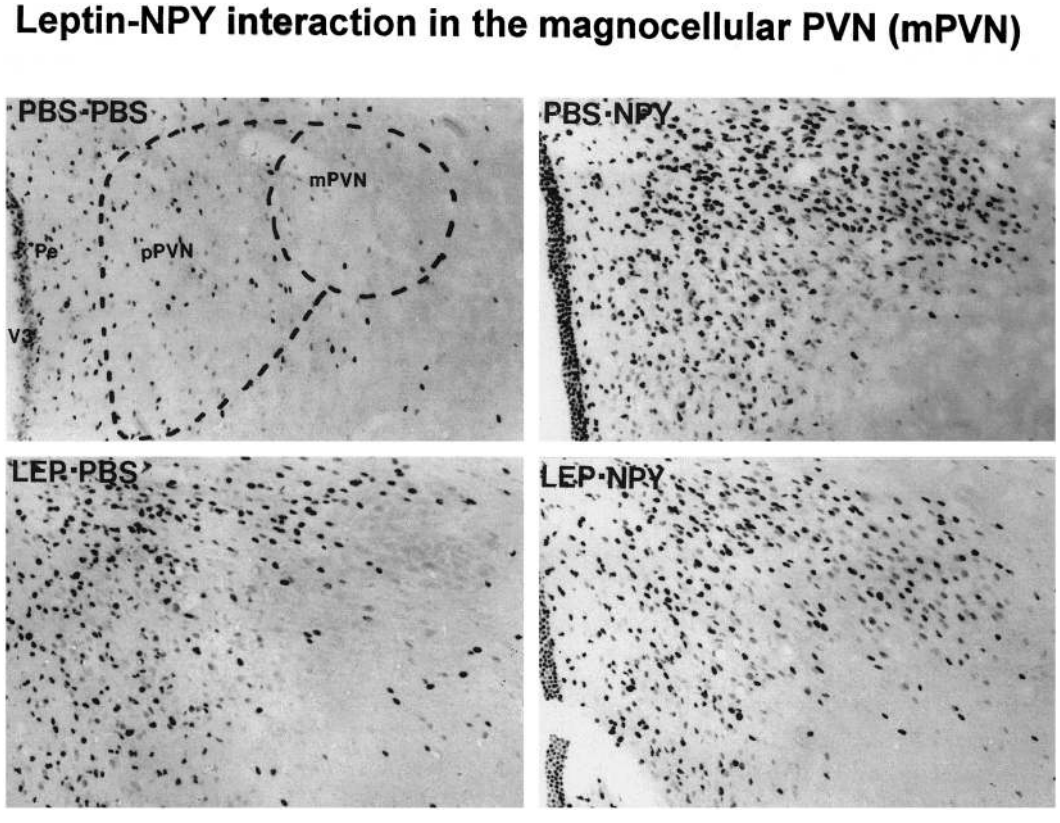


FIG. 3. Photomicrograph showing c-FOS-like immunopositive cells in the parvicellular and magnocellular regions of the PVN (pPVN and mPVN, respectively) of an intraventricularly PBS-injected control (*top left*), NPY- injected rat (*top right*), leptin-injected rat (*bottom left*), and leptin + NPY-injected rat (*bottom right*). Whereas leptin on its own failed to activate c-FOS, it (LEP+PBS) significantly decreased NPY-induced c-FOS immunoreactivity selectively in the mPVN and food-intake (LEP+NPY). $P_e$, Periventricular area; $V_3$, third ventricle. [Reproduced with permission from M. Yokosuka *et al.*: *Physiol Behav* 64:331–338, 1998 (90) with permission from Elsevier Science].

34), and NPY $Y_1$ receptors are located in POMC- and NPY-producing cells (166). Seemingly, NPY released in the ARC not only autoregulates its own release but also the release of other orexigenic signals.

*c. Is NPY a physiological appetite transducer?:* Among the known orexigenic signals, NPY is apparently the only messenger molecule that can be considered a physiological appetite transducer in the brain. Critical evidence to satisfy the basic criteria in support of this view is as follows: 1) Central administration of NPY stimulated feeding not only in satiated rats, but it also rapidly enhanced ongoing feeding (169). 2) As expected of a physiological signal, augmentation in food intake was dose dependent, and the response followed a bell-shaped curve; higher doses were less effective and produced a different pattern of feeding reflected in altered local eating rate and time spent eating (137). 3) Continuous central infusion of NPY reproduced the characteristic episodic nocturnal feeding pattern (170) and various components of feeding behavior, such as cumulative food intake, number of feeding episodes, mean episode length, total time spent eating, eating rate (g/min), and interepisode interval, were affected in a dose dependent manner. These similarities between the normally occurring nocturnal feeding behavior and that reproduced by NPY in satiated rats reinforced the view that intermittent nocturnal feeding in rats is chemically coded and presumably dependent upon increased NPY release and action (171). 4) This assumption was underscored by the finding that immunoneutralization of NPY during the dark phase of the light-dark cycle blocked nocturnal feeding (172). 5) Enhanced NPY release, selectively in the PVN, was temporally correlated with food consumption in rats maintained on a scheduled feeding regimen (106). The rate of NPY release in these rats rose just before onset of feeding and progressively decreased as the animal consumed food. Further, these rats continued to hypersecrete NPY if food was withheld, clearly pointing to the participation of NPY in sustaining appetite. 6) Fasting markedly augmented NPY release in the PVN both in vivo and *in vitro* (106, 107). 7) Fasting and food restriction augmented NPY $Y_1$ receptor mRNA in the hypothalamus (Ref. 173; Fig. 4). 8) Hyperphagia and obesity in several genetic and experimental models were associated with modifications in NPYergic signaling. Genetically obese ob/ob mice, db/db mice, and fatty Zucker rats (fa/fa) exhibited increased prepro-NPY mRNA in the ARC (174–176) and NPY levels in various hypothalamic sites and release in the PVN. 9) Augmented NPYergic signaling in the hypothalamus, as evidenced by increases in ARC NPY gene expression and PVN NPY levels (177–179) and release (179–181), preceded the onset of hyperphagia induced experimentally by streptozotocin (STZ) treatment and upon extension of the duration of diabetes in these rats; the augmented NPY response spread from the PVN to practically all hypothalamic sites (177–181). 10) Increased energy demands during lactation, apparently met by hyperphagia, were accompanied by increased NPYergic signaling in the ARC-PVN axis (15, 182). 11) Selective $Y_1$ and $Y_5$ receptor antagonists attenuated both nocturnal feeding and that induced by fasting (18, 19, 150–152, 155, 158, 159). 12) Studies conducted in mice lacking either $Y_1$ or $Y_5$ receptors also affirm the involvement of NPY in food intake and obesity. In mice

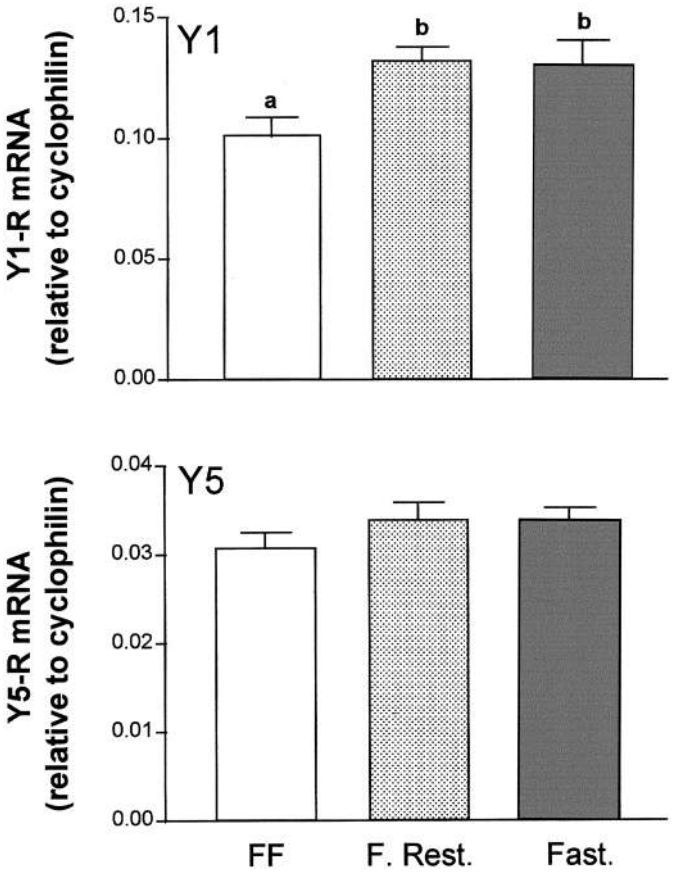


FIG. 4. Up-regulation of NPY $Y_1$ receptor mRNA in the hypothalamus of food-deprived (Fast) rats and in rats maintained on food restriction (F.rest) as compared with free-fed (FF) rats. *Dissimilar superscripts* are significantly different ($P < 0.05$). [Adapted with permission from B. Xu *et al.: Regul Pept* 75–76:391–395, 1998 (173)].

deficient in expression of the $Y_1$ receptor subtype, daily food intake and intake in response to fasting was significantly reduced (183). Interestingly, these mice displayed a late onset of increase in body weight. Our observations that fasting normally increases the availability of $Y_1R$ (173) and selective $Y_1$ antagonists inhibit feeding (18, 19), taken together with the results from $Y_1$ receptor knockout mice, clearly underscore a role of NPY in control of food intake and obesity, a conclusion in line with earlier results (184). With respect to involvement of other NPY receptor subtypes, it has been shown that $Y_5R$-null mice feed normally early on, display normal feeding response to exogenous NPY, but show late-onset obesity due to increased body weight (185). These observations are consistent with NPY's participation in stimulation of feeding and reveal that $Y_1$ and $Y_5R$ are involved but, as documented earlier (18, 19, 184), signaling via $Y_1R$ may be a prominent player in mediating feeding induced by NPY. In view of the several recent reports (186–188), it seems that either both or another subtype closely related to $Y_1$ and $Y_5$ subtypes may mediate the appetite-stimulating effects of NPY. An exciting new insight from these studies is that not only a partial deficiency in NPY availability induced experimentally in rats (Refs. 18, 19, and 63; see *Section III.A.4b*) but now a complete deficiency of either $Y_1$ or $Y_5$ receptor subtypes results in hyperphagia and obesity. Since in NPY-

deficient rats, contribution of increased responsiveness to NPY due to up-regulation of $Y_1R$ (18, 19, 63) and up-regulation of GAL and GAL responsiveness have been demonstrated (64, 66, 67), a similar pattern of shifts in signal transduction and orexigenic signals responsible for late-onset obesity is highly likely in $Y_1$ and $Y_5$ receptor mutant mice.

*2. Regulation of hypothalamic NPY secretion.* As would be expected of a naturally occurring appetite transducer, multiple neural and hormonal signals affect NPYergic signaling in the hypothalamus. In the majority of studies, NPYergic signaling was evaluated by analysis of either steady-state NPY levels in discrete hypothalamic sites or prepro-NPY mRNA levels in the ARC during the lights-on period when rats do not normally eat. NPY release in discrete hypothalamic sites has been examined only occasionally in correlation with feeding behavior (106, 107, 174). Despite these shortcomings, the accumulated information, as summarized below, indicates that regulation of NPY secretion in the daily management of ingestive behavior by the hypothalamus is complex and multifactorial.

*a. Neural control of NPY secretion:*

*i) Circadian clock.* Since eating in rats occurs primarily during the dark phase, a temporally correlated pattern of changes in hypothalamic NPY levels, release, and synthesis was anticipated. Akabayashi *et al.* (131) reported increased prepro-NPY mRNA in the basal hypothalamus immediately preceding onset of the dark phase, followed by a precipitous drop during the nocturnal period of feeding. However, NPY levels remained elevated in the large dorsomedial hypothalamic area, a part of neural tissue containing PVN and several other adjoining nuclei, during the dark phase. A detailed reexamination by Xu *et al.* (120, 121, 123) revealed that prepro-NPY mRNA levels increased during the lights-on phase between 0700–1100 h, and then decreased and remained in the basal range throughout the entire dark phase (Fig. 2). Evidently, a transient elevation in NPY gene expression occurred several hours before the onset of nocturnal feeding (Fig. 2). These findings implied that an independent neural component, driven by the circadian clock, regulated this daily rhythm in gene expression (120, 121, 123). Indeed, evidence showed that up-regulation of ARC NPY gene expression, brought about by food restriction to 4 h during the lights-on period, was sustained throughout the 24-h period despite daily changes in the light-dark cycle (121, 123, 189). This abolition of the daily rhythm in the ARC NPY gene expression suggests uncoupling from the photoperiodic signals emanating from the SCN. The neural pathways that transmit information generated by changes in the pattern of food availability are currently unknown.

*ii) Serotonin (5-HT).* Guy *et al.* (190) described nonsynaptic appositions between 5-HT nerve terminals and immunoreactive NPY perikarya and dendrites in the ARC. Subsequently, Dube *et al.* (191) showed that fenfluramine, which increases serotoninergic signaling and reduces food intake, decreased NPY levels without acutely changing release in the PVN of food-deprived rats. A possible inhibitory influence of 5-HT on NPYergic signaling was later affirmed by a series of studies by Williams and co-workers (192, 193). Pharmacological agents that activated 5-HT output inhibited NPY levels and release in the PVN concurrently with anorexia. Conversely, 5-HT antagonists stimulated feeding in conjunction with increased NPY levels in the ARC and PVN. Although these morphological and pharmacological reports collectively imply an inhibitory role of 5-HT on NPY release, the physiological relevance of the communication between serotoninergic and NPYergic signalings in the daily patterning of food intake warrants further investigation.

*3. Hormonal regulation of NPY secretion.* Because of its strategic distribution, circulating signals are in direct communication with the NPY system in the hypothalamus. Steroids originating from the gonads and adrenal cortex exert a modulatory influence on NPY synthesis and release (194–199). In addition, metabolic signals relayed by the circulating peptide hormones, insulin and leptin, and cytokines can affect the NPY system directly and also are suspected to cross the blood-brain barrier and gain access to the cerebrospinal fluid through the circumventricular organs (Refs. 16, 17, 200, 201; see *Sections III.A* and *V*).

*a. Gonadal steroids:* It has been known for a long time that gonadal steroids modulate food intake and body weight gain in rodents and other mammals (15). In the female, ovariectomy generally increased food consumption and body weight gain, and estrogen replacement reinstated the normal pattern of daily intake (15). Gonadal steroids promoted NPY neurosecretion in the hypothalamus (194–196, 202) as evidenced by the findings that gonadectomy decreased prepro-NPY mRNA levels in the ARC, and testosterone replacement reinstated gene expression and NPY levels in selected hypothalamic sites in male rats. In ovariectomized female rats, estrogen treatment exerted a bimodal effect. Short-term treatment induced daily changes in NPY gene expression in the ARC with a rise in the afternoon sustained for a few hours, a response reminiscent of the pattern of gene expression seen on proestrus in cycling rats (195, 203). In contrast, a prolonged uninterrupted increase in estradiol in physiological levels decreased NPY levels selectively in the PVN and PFH and decreased NPY release from the PVN (204). These results were interpreted to imply that the anorectic effects of estrogen were mediated by decreased NPY release from the PVN (204). Since NPY-producing neurons possess estrogen receptors (194), it is highly likely that chronic estrogen action suppressed both NPY synthesis in the ARC and release in the PVN. Baskin *et al.* (205) also reported recently that estrogen inhibited the fasting-induced increase in hypothalamic NPY gene expression. Evidently, an underlying role of estrogen is to inhibit food intake via direct action on the NPY pathway in the ARC-PVN of female rats.

*b. Adrenal glucocorticoids:* Unlike the hypothalamo-pituitary-gonadal axis, the interaction of the hypothalamic-pituitary-adrenal axis with the NPY system, particularly in relation to nocturnal feeding in the rat, has been unclear despite the concerted efforts of a number of investigators. Experimental evidence invoking the existence of a feedback relationship between the adrenal cortex and hypothalamic NPY system are the following: NPY-producing neurons are synaptically linked with CRH neurons in the PVN (206). Microinjection of NPY into the PVN rapidly activated pituitary ACTH and adrenal corticosterone secretion, suggesting that

this communication is excitatory in nature (207). NPY neurons in the ARC are rich in glucocorticoid receptors (197, 198), and treatment of adrenalectomized rats with glucocorticoids increased NPY gene expression in the ARC in association with increased food intake and body weight (197, 199, 208). On the other hand, unlike the effects of gonadectomy, it is intriguing that adrenalectomy either decreased or elicited no effect on hypothalamic NPY levels and NPY gene expression in the ARC (209–211). Adrenalectomy failed to affect the time of onset and pattern of nocturnal feeding, leading one to infer that NPY-induced nocturnal feeding is not tightly coupled to the daily rhythm in the hypothalamo-pituitary-adrenal axis. Further, in the absence of circulating corticosterone, rats showed only a small decrease in cumulative food intake, and NPY-induced feeding under these conditions was minimally attenuated (211). These unremarkable effects on food intake were attributable to the generally depressed metabolic responses of adrenalectomized animals. Also, contrary to previous reports (209–212), recent studies showed that the fasting-induced up-regulation of hypothalamic NPY gene expression was not dependent upon adrenal glucocorticoids (213). Evidently, NPY can stimulate the hypothalamo-pituitary-adrenal axis (207), but whether glucocorticoids play a role in governing NPY output for nocturnal feeding is uncertain.

*c. Insulin:* Although it has been suggested, but not completely proven, for a long time that pancreatic insulin may act centrally to regulate body weight by restraining food intake (200, 201), the hypothalamic signaling pathway underlying the anorexigenic effects of insulin was unknown. The reports that insulinopenia produced by STZ increased NPY levels in various hypothalamic sites in association with hyperphagia, and insulin replacement normalized NPY levels and blocked hyperphagia (176–179, 214, 215), provided the breakthrough in firming the concept that peripheral signals exert an inhibitory influence on hypothalamic orexigenic pathways. NPY gene expression and NPY levels in the PVN increased before the onset of hyperphagia in STZ-treated rats (181). Concurrently, NPY release increased selectively in the PVN (180). Importantly, insulin replacement, in doses that did not affect hyperglycemia in STZ-treated rats, markedly decreased NPY release in the PVN (180). Thus, it is highly likely that insulin per se exerted a regulatory influence on NPY release in the PVN. The site of insulin action is apparently the PVN because insulin inhibited NPY release from the PVN *in vitro* (216). Whether insulin inhibits NPY release directly from NPY nerve terminals or through interneurons has not been delineated. Further, central infusion of insulin attenuated the fasting-induced increase in NPY gene expression (201). Since the effects of insulin on PVN NPY release were rapid (216), it is highly likely that attenuation of the ARC NPY gene expression by central infusion of insulin may be secondary to inhibition of NPY release from axon terminals in the PVN (216). A direct inhibitory action of insulin at the NPY perikaryal level is also doubtful because central infusion of insulin failed to activate c-FOS in the ARC (217), and insulin receptor immunoreactivity (201) has not yet been visualized on NPY neurons in the ARC. These findings are noteworthy when compared with the response elicited by leptin, which also inhibited NPY synthesis (see *Section V*), but readily stimulated c-FOS expression in several hypothalamic sites (89, 90). Nevertheless, there is a general consensus that insulin deficiency results in NPY hypersecretion which, in turn, induces hyperphagia. The physiological relevance of this postulated restraint of NPY release by insulin in nocturnal feeding is largely unclear. Since insulin rapidly inhibited NPY release from the PVN nerve terminals, it is possible that the postprandial insulin rise (216) exerts a restraint, along with leptin (Fig. 2; Refs. 121 and 123), on NPY release in the PVN to prevent hyperphagia. This nocturnal restraint on ongoing feeding, we believe, constitutes one of the mechanisms enforcing set point in body weight.

*d. Cytokines:* During infection, CNS injury, or other pathological conditions, anorexia leading to a loss in body weight is manifested (9–11). Recent evidence points to a possible relationship between increased circulating levels of cytokines and hypothalamic NPYergic signaling in induction of anorexia during the pathological state. Interleukin-1 was shown to decrease food intake probably by interfering with NPY's ability to stimulate feeding (10). Ciliary neurotrophic factor (CNTF), another cytokine, is a neurotrophic factor, but systemic and central injections of CNTF produced severe anorexia (218, 219). Xu *et al.* (219) reported that CNTF decreased the availability of NPY for release by suppressing NPY gene expression in the ARC. In addition, CNTF markedly inhibited NPY-induced feeding by decreasing $Y_1$ abundance (173). Seemingly, CNTF, and possibly other anorectic cytokines, acts within the hypothalamus through their respective receptor systems to decrease NPY availability and also to suppress the postsynaptic signal transduction processor. A consequence of the interaction of cytokines with the NPY system, thus, is anorexia and loss in body weight during pathological conditions.

*4. Role of NPY in the etiology of hyperphagia and obesity in rodents.* There are several genetic and experimentally induced models of obesity in rodents (6, 220–222). Although a hallmark of obesity is an abnormal increase in body weight attributable to multiple metabolic disturbances, partly dependent upon an imbalance in the endocrine and autonomic systems, a primary causal factor is the unrelenting drive to eat. The unregulated feeding pattern manifests either throughout the day or selectively during the dark phase. Based on the new insight that NPY is a primary appetite transducer within the interconnected orexigenic network in the hypothalamus, the possibility that modifications in NPYergic signaling may underlie hyperphagia in various models of obesity has been explored extensively.

*a. Genetic models of obesity:* Evidence accumulated to date clearly showed that in genetically obese ob/ob and *db/db* mice, hyperphagia may be attributed to unabated NPY output in the hypothalamus (174–176, 223–225). Whereas the increase in hypothalamic NPYergic signaling in *ob/ob* mice is due to leptin deficiency (16, 17, 226–230), in *db/db* mice a defect in leptin signal transduction due to a point mutation in the leptin receptor is the causal factor (231–234). Similarly, in fatty Zucker rats (*fa/fa*), hypothalamic NPYergic signaling is augmented as evidenced by increased NPY levels in various hypothalamic sites, NPY gene expression in the ARC, and NPY release in the PVN (174, 176–178). The fact that

immunoneutralization of NPY in Zucker rats suppressed feeding affirmed an important role of NPY in this model of hyperphagia (235). Leptin resistance is believed to underlie NPY hyperactivity in Zucker rats (236). Also, in corpulent (*cp/cp*) JCR-LA obese rats, there is evidence of overactive NPYergic signaling in the hypothalamus (237).

*b. Experimental models of obesity:* In rats, hyperphagia and abnormal body weight gain leading to obesity can be experimentally induced by disruption of neural pathways either by localized lesions in the hypothalamus, transection of neural pathways in the basal hypothalamus, or by injection of neurotoxins in hypothalamic and extrahypothalamic sites (24–30, 238). A concerted effort in our laboratory, as summarized below, aimed at elucidating the neurochemical basis of experimentally induced hyperphagia and obesity, revealed disturbances in leptin-NPY signaling (see also *Section V*).

*i) Hyperphagia and obesity due to disruption in VMH signaling.* It has been known for more than 50 yr that lesions in the VMH are associated with hyperphagia and abnormal body weight gain in rodents and humans (24–26, 29). In rats, VMH lesions produced rapid and longlasting hyperphagia resulting in overt obesity (24–26). Contrary to expectations, prepro-NPY mRNA levels in the ARC and NPY levels and release from the PVN were markedly suppressed in these rats (1, 12, 145, 146). This diminution in hypothalamic NPYergic signaling in hyperphagic VMH-lesioned rats contrasts with the hyperactive NPY system prevailing in genetic models of obesity in rodents (174–176, 214) and in diabetic rats (177–181). Interestingly, despite the low levels of NPY secretion, hyperphagia was dependent upon NPY because immunoneutralization of NPY completely suppressed feeding in these rats (239).

In another model in which neural signaling within the VMN was disrupted by microinjection of the neurotoxin colchicine, transient hyperphagia, lasting only for 4–5 days developed rapidly with an attendant increase in body weight (18, 19, 63, 240). As in the VMH-lesioned rats, NPYergic signaling, as demonstrated by diminution in NPY mRNA in the ARC (18, 19, 63) and NPY release in the PVN (65), diminished immediately after colchicine injection. In these rats, hyperphagia was found to be, in part, due to increased sensitivity to NPY (Fig. 5) and abundance of $Y_1$ receptors in the hypothalamus, blockade of which by $Y_1$ receptor antagonist inhibited feeding (18, 19, 63). The role of NPY $Y_5$ receptors in this paradigm is not clear. In addition, even this temporary disruption in VMN function rendered rats resistant to the action of leptin (1, 18, 19, 63). Seemingly, either one of the targets of leptin feedback resides in the VMN or alternatively, an intact VMN is required for relay of leptin feedback information to sites outside the VMN. In the other models of obesity, such as that induced by gold thioglucose in mice or by postnatal treatment of rats with monosodium glutamate, hypothalamic NPY gene expression and NPY levels in the PVN were similarly suppressed, and feeding in response to NPY was increased (241, 242).

A further evaluation recently showed that disruption of signaling by colchicine (COL) in the VMN augmented NPY gene expression in PVN and DMN, the nuclei that normally either do not or only minimally express NPY mRNA. Interestingly, these rats also start to produce excessive GAL and display increased responsiveness to GAL (63–68).

Taken together, evidence from genetic and experimental models of rodent obesity showed that both over- and underexpression of NPY resulted in unregulated phagia and body weight gain (1). In the case of low NPY abundance, hyperphagia is seemingly due to multiple factors brought

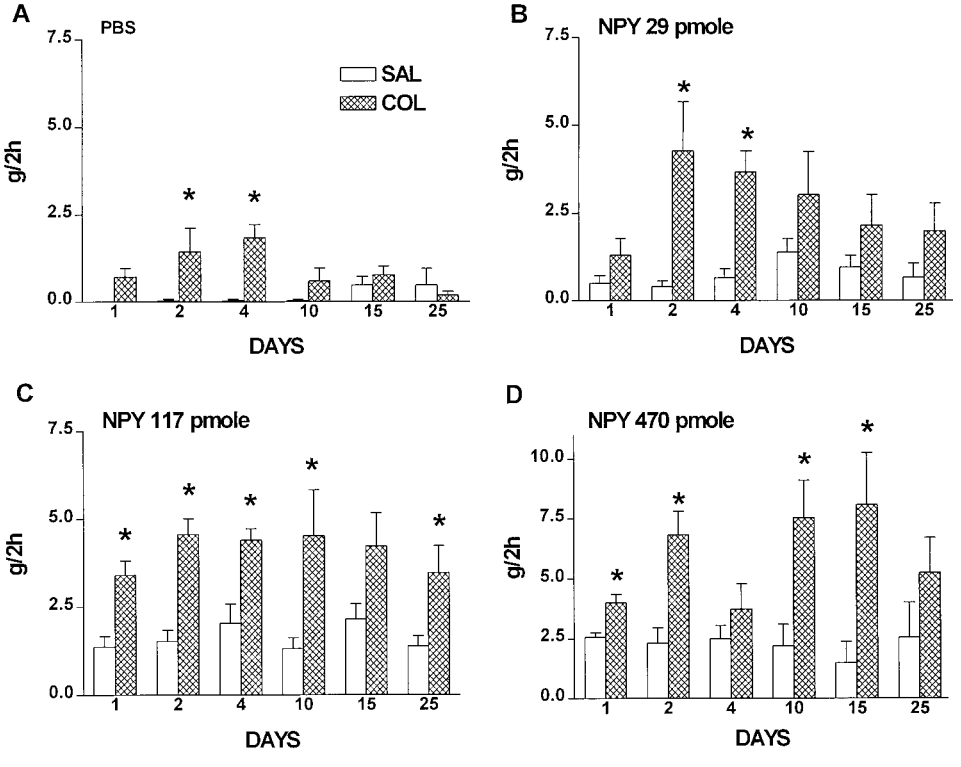


FIG. 5. Increased sensitivity to intracerebroventricular administration of NPY in stimulation of food intake in hyperphagic rats that received colchicine (COL) in the VMN. Each panel compares data from cholchicine- and saline (SAL)-injected rats in response to vehicle (PBS) or NPY (three doses) at various intervals post colchicine. Food intake in response to NPY was consistently greater in colchicine-treated rats *vs.* SAL-treated rats on the same day. Note that the increased sensitivity to NPY occurred concomitantly with reduced hypothalamic NPY synthesis and release in the PVN (see text). [Reproduced with permission from P.S. Kalra *et al.: Regul Pept* 72:121–130, 1997 (63)].

about by a shift in the normal pattern of NPYergic signaling in the ARN. These include 1) a rapid increase in NPY $Y_1$ receptors in the hypothalamus leading to increased sensitivity to NPY (18, 19, 63); 2) concurrent development of leptin resistance in these rats, a hypothesis in line with the hypothesis that a normally functioning VMH is necessary for the central inhibitory effect of leptin on food intake (1, 18, 19); 3) up-regulation of NPY in novel hypothalamic sites; and 4) increase in galaninergic signaling.

*ii) Hyperphagia and obesity due to interruption of NE input from the BS.* Microinjection of the neurotoxin 6-OHDA into the ventral NE bundle at the level of trochlear or oculomotor nuclei induced hyperphagia and obesity (238, 243). Overt hyperphagia manifesting several days after 6-OHDA injection was long lasting and accompanied by increased rate of body weight gain (238). Interestingly, the disruption in NPY-leptin signaling in these rats was markedly different from that seen in the hyperphagia and obesity syndrome produced by either electrolytic lesions in the VMH (1, 12, 145, 146) or colchicine injection (18, 19, 63) in the VMN. The dark-phase hyperphagia in 6-OHDA-treated rats was accompanied by two modifications in NPY-leptin secretion. The normal nocturnal increase in leptin secretion after eating (Fig. 2) was abolished by interruption of catecholaminergic afferents to the hypothalamus (62). Further, it was shown that diminution in leptin feedback was responsible for the increase in nocturnal NPY secretion, $Y_5$ receptor mRNA, and hyperphagia (62).

Thus, analysis of the etiology of hyperphagia and obesity produced experimentally revealed a number of loci in NPY-ergic signaling within ARN that are vulnerable to disruptive insults. A provocative new concept emerging from these efforts is that both low and high abundance of NPY result in hyperphagia and obesity (1, 18, 19, 63). The evidence clearly showed that increased receptor abundance, NPY receptor sensitivity, and up-regulation of NPY in novel hypothalamic sites, together with increased availability of other orexigenic signals, such as GAL, underlie hyperphagia. Shifts in the leptin secretion pattern and development of leptin resistance also contribute toward this altered hyperphagia, producing peptidergic signaling.

### *B. Galanin*

Galanin (GAL), a 29-amino acid peptide, has been studied extensively for its appetite-stimulating action in the brain. Intraventricular or intrahypothalamic injection of GAL stimulated feeding in satiated rats (71–73, 244–248), and it appears that receptive sites of GAL may be widely distributed in the rat brain. Microinjection of GAL in the PVN, LH, VMN, and central nucleus of the amygdala stimulated feeding in rats. As compared with NPY, GAL-induced feeding is less remarkable, lasting for only about 30 min with augmented response apparent at the onset of the dark phase (71–73, 246, 248).

Unlike the discrete distribution of NPY-producing cells (21, 140), several subpopulations of GAL-producing neurons exist in the hypothalamus that innervate most of the hypothalamus (45). A close anatomical and functional relationship exists between GAL and other orexigenic signals producing neurons in the rat brain. NPY-producing neurons were shown to be in direct communication with GAL-producing neurons, especially in the ARC and PVN (33). Thus, GAL may, in part, mediate NPY-induced feeding (1). Further, GAL-immunopositive nerve terminals establish synaptic links with POMC containing dendrites and soma in the ARC (35), raising the possibility that GAL may stimulate the release of $\beta$-END, which also stimulates ingestive behavior (see *Section III.C*). Pretreatment of rats with naloxone (NAL), an opiate receptor antagonist, markedly attenuated GAL-induced feeding (246). However, because NAL pretreatment failed to completely block the feeding response, it is quite likely that GAL directly stimulates feeding by activating GAL receptors in hypothalamic sites (248–251). Involvement of NE in GAL-induced feeding is suggested by the reports that GAL stimulated NE release in the PVN, and blockade of $\alpha_2$-adrenergic receptors with rauwolscine attenuated the feeding stimulated by GAL (244). Furthermore, the two orexigenic signals are coproduced in a subpopulation of neurons in the BS that project into various hypothalamic sites, including the PVN (22), and stimulation of modest feeding under the influence of GAL resembles that induced by NE (72, 73). Thus, release of NE in the PVN and neighboring sites by GAL from nerve terminals emanating from perikarya in the BS may mediate GAL-induced feeding (22). Undoubtedly, feeding elicited by GAL involves an interplay among various orexigenic signals, as documented by the existence of communication lines consisting of NPY→GAL→$\beta$-END and GAL→NE.

Whether GAL constitutes an important orexigenic signal in the daily pattern of feeding has not been clearly established. GAL-producing cells are localized in discrete subpopulations in the ARC, DMN, and PVN, but their participation in normal feeding behavior in the rat is not known. Similarly, high-affinity GAL receptors are distributed in multiple sites including those where GAL microinjection stimulated feeding (252, 253). However, GAL receptor antagonists, including M-40 that readily inhibited GAL-induced feeding, failed to suppress normal feeding in several behavioral paradigms (46, 248, 249). Infusion of GAL antisense oligonucleotides in the PVN inhibited feeding (254) but, unlike NPY, continuous GAL infusion was ineffective in increasing food intake and body weight gain (255).

Attempts to establish a correlation between the daily feeding pattern and changes in GAL synthesis and release in the hypothalamus have not been instructive. Increased appetite, evoked by either restricted feeding or fasting, decreased GAL gene expression in the ARC (256–259). A small increase in GAL levels in the lateral portions of the pPVN was observed 3 h into the dark phase (118); the physiological significance of this in normal nocturnal feeding is unknown. Interestingly, the daily increases in GAL gene expression in the hypothalamus temporally coincide with those in hypothalamic POMC mRNA but lag slightly behind those in NPY (Fig. 2; Refs. 120, 121, and 123). Also, unlike NPY (174–176), GAL levels in the PVN and other hypothalamic sites do not correlate with hyperphagia in Zucker fatty rats (257). Consequently, additional studies are warranted to define the precise physiological role of GAL in the daily pattern of feeding behavior.

*C. Endogenous opioid peptides*

Although it has been known for a long time that opiates promote phagia (260, 261), interest in the roles of endogenous opioid peptides (EOP) gained momentum after the isolation and characterization of the three biologically active opioids—β-END, dynorphin A (DYN), and enkephalins—in the hypothalamus (51–53). The single-copy POMC gene encodes the POMC precursor PP, which yields the opioid, β-END, and the nonopioid peptides, ACTH and αMSH (51–53). Within the hypothalamus, POMC neurons, localized exclusively in the ARC, innervate the VMN, PVN, DMN, and other areas of the hypothalamus (43, 44), where microinjection of β-END and opiate agonists that bind to the μ-receptor subtype stimulate feeding (13, 23, 74). Neurons producing the two pentapeptides, methionine-enkephalin (met-ENK) and leucine-enkephalin (leu-ENK), are more widely distributed in the hypothalamus, and discrete populations of leu-ENK- or met-ENK-immunopositive perikarya have been visualized in feeding-relevant sites, such as the ARC, VMN, DMN, and PVN, sites that are also richly innervated by ENK-immunopositive fibers (13, 42, 44). Although ENK were ineffective on their own, the long-acting analogs stimulated feeding, possibly involving the δ-receptor subtype (13, 261). Central injection of the third hypothalamic opioid, dynorphin A 1–17, derived from the precursor prodynorphin, stimulated feeding by activating the κ-opiate receptor subtype. DYN-producing cells are also found in various regions of the hypothalamus, including the ARC and PVN (13, 44, 261, 262).

A critical evaluation of food intake induced by various EOP showed that opioid-evoked feeding, unlike that evoked by NPY, is generally short lived and relatively modest (261). Extensive evidence from clinical and animal experiments suggested that opiate receptor antagonists, especially the μ- and κ-antagonists, decreased food intake (13, 262–266). However, whereas fasting and diabetes increased NPY mRNA, POMC mRNA was decreased, but DYN mRNA was augmented in the PVN (258, 267). The daily rhythm in POMC gene expression was similar to that of GAL but lagged behind the rhythm in NPY gene expression by a few hours (Fig. 2; Refs. 120, 121, and 123).

Although a large body of evidence concurs with the orexigenic nature of EOP, especially β-END, whether these peptides subserve a relevant physiological role or represent redundant pathways in the daily management of ingestive behavior remains to be experimentally documented. However, the recent anatomical studies establishing morphological links among β-END, GAL, and NPY and between GABA and β-END immunoreactive pathways in the hypothalamus are highly suggestive of a role of β-END within the orexigenic network (1, 33–37). Horvath *et al.* (34) showed that NPY immunopositive terminals established synaptic contacts with β-END-containing dendrites and soma, suggesting that NPY may induce feeding directly on its own and also by stimulating β-END in relevant sites such as the PVN. Indeed, NPY was shown to stimulate β-END release in the hypothalamus (56), and NAL pretreatment attenuated feeding in response to NPY (13, 261). On the other hand, NPY decreased POMC mRNA levels in the ARC (268) and the opiate receptor antagonist, NAL, increased prepro-NPY mRNA in the ARC and increased NPY levels in the DMH (265). Morphological links between β-END and GAL systems in the ARC and between GABA and β-END in the ARC and PVN are suggestive of a role in regulation of GAL and GABA in the release of β-END (33–37). Pharmacological evidence shows that opiate receptor antagonists decreased feeding stimulated by GAL (246) and by the $GABA_A$ receptor agonist, muscimol (13, 269). Collectively, these observations suggest that β-END pathways may represent an important signaling modality in the operation of the interconnected orexigenic network (1).

*D. Melanin-concentrating hormone*

A population of neurons in the zona incerta and LH produce the 19-amino acid peptide, melanin-concentrating hormone (MCH), and project into several hypothalamic and limbic areas (270–273). Qu *et al.* (274) reported potentiation of ongoing nocturnal feeding for 4–6 h by MCH administration at the onset of the dark phase. Further studies revealed several parallels between MCH and NPY systems in the hypothalamus. MCH augmented ongoing feeding (274), fasting stimulated MCH gene expression in the hypothalamus, and MCH mRNA was elevated in genetically obese *ob/ob* mice (274). MCH also stimulated the hypothalamo-pituitary-adrenal axis (275). These similarities raised the intriguing possibility that MCH may be an additional orexigenic signal that either independently or together with NPY participates in the hypothalamic regulation of energy homeostasis. However, this assumption must await further experimental validation because Presse *et al.* (276) reported potent anorectic effects on nighttime food intake and suppression of body weight by extremely low doses of MCH. Similarly, microinjection of MCH into the zona incerta-LH region reduced feeding (276, 277). In contrast, Rossi *et al.* (278) confirmed the orexigenic effects of MCH in rats, but relative to NPY, MCH-induced feeding was small and of short duration. Further, despite the acute stimulatory effects of MCH, cumulative 24-h intake was unaffected, and repeated daily injections stimulated food intake for a few days without changing the body weight. Interestingly, small lesions in the VMN that stimulated MCH gene expression in the hypothalamus failed to evoke hyperphagia (279, 280). Because MCH stimulated feeding in normal satiated rats, a modulatory role of MCH in situations of augmented appetite, as that during fasting or in genetically obese *ob/ob* mice, may be envisioned.

*E. Amino acids: glutamate and γ-aminobutyric acid*

The EAA glutamate and the inhibitory amino acid γ-aminobutyric acid (GABA) are produced in various hypothalamic sites and are the most abundant neurotransmitters in the hypothalamus (47–50). Intriguingly, both neurotransmitters stimulated feeding in the rat. *N*-methyl-D-aspartic acid (NMDA), the glutamate receptor agonist, stimulated immediate and transient feeding lasting for about 10 min only when microinjected into the LH (281, 282). It is possible that EAA receptors involved in excitation of feeding are located selectively in the LH and are in a position to impact the

orexigenic peptidergic network in the hypothalamus. Thus, excitation with NMDA of neural pathways originating and/or traversing the LH may stimulate the release of orexigenic signals such as NPY, GAL, opioids, and orexins; however, since NMDA-induced feeding was short lived as compared with that induced by peptides, the possibility that neuropeptides mediate the NMDA-induced feeding is highly unlikely. Therefore, the physiological relevance of the orexigenic action of EAA in the integration of ingestive behavior remains to be sorted out.

In contrast, microinjection of muscimol, the $GABA_A$ receptor agonist, into several hypothalamic sites, *e.g.*, VMN, DMN and PVN, readily stimulated feeding lasting for 30 min (74–77, 269). Since these hypothalamic sites also respond to other orexigenic peptidergic signals, such as NPY, GAL, $\beta$-END, and dynorphin, it is likely that $GABA_A$ receptors and receptors for these orexigenic peptides may be localized on the same target cells.

Recent investigations of the morphological and functional relationship of GABA with other orexigenic signals shed light on the involvement of the hypothalamic GABAergic network in stimulation of appetite in the rat. Horvath *et al.* (283) showed that GABAergic fibers form synaptic contacts with those $\beta$-END-containing neurons in the ARC that project into the diverse sites, raising the likelihood that GABAergic synapses regulate the output of POMC-derived peptides. Additionally, observations that the $\beta$-subunit of $GABA_A$ receptors was localized within the POMC-immunopositive neurons (284), and muscimol and GABA inhibited $\alpha$MSH release and POMC gene expression (285), are suggestive of an interplay of GABA with other orexigenic and anorexigenic signals.

Recent observations that ascribe a novel physiological role to GABA are coexpression of GABA in NPY- and GAL-producing subpopulations of neurons in the ARC (36). Further, a large number of NPY and GABA coexpressing neurons project into the PVN (37, 38). Because injections of NPY and muscimol either intraventricularly or directly into the PVN elicited a synergistic feeding response (37, 38), it is likely that corelease of NPY and GABA in the PVN and neighboring sites may amplify feeding. These revelations, coupled with the additional possibility that NPY- and GABA-coexpressing cells in the ARC regulate $\alpha$MSH (285) and $\beta$-END (56) release from POMC neurons and other anorexigenic signals, clearly favor the operation of an interconnected orexigenic network in which peptides and GABA are intimately involved in stimulation of appetite.

On the other hand, the excitatory effects of GABA on feeding run counter to the prevailing notion that GABA mediates inhibitory synaptic transmission in the brain (47). Therefore, one cannot exclude the alternate possibility that GABA may inhibit a tonic restraint to evoke feeding either on its own or in conjunction with NPY and other orexigenic signals (36–38). We suggest that GABA may curtail a tonic restraint both by decreasing $\alpha$MSH release and altering the response at target sites, rendering them highly responsive to NPY. This two-prong action of coreleased NPY and GABA thus serves to amplify feeding (Fig. 7 and *Section IV*). It is also important to note that biphasic responses to GABA, an initial inhibition followed by excitation, are observed either when large amounts of GABA are employed or when inhibitory synapses are activated at high frequency, causing depolarization of postsynaptic contacts to trigger an action potential (286, 287). Further studies devoted to understanding the precise mechanism of GABA involvement are needed to clarify its role in the daily patterning of feeding behavior and in the peptidergic orexigenic network.

### *F. Hypocretins/orexins*

Recently, a new class of orexigenic peptides was isolated and characterized in two separate laboratories. De Lecea *et al.* (288) reported that neurons producing a pair of peptides in the hypothalamus, called hypocretins I and II (Hcrt1 and Hcrt2), were localized in clusters in the dorsal and lateral hypothalamic area and PFH. Immunoreactive axons emanating from these cells innervated various forebrain structures anteriorly, such as the ARC, paraventricular nucleus of the thalamus, POA and the septal nuclei, and caudally the locus coeruleus and a few other sites in the BS. Almost simultaneously, Sakurai *et al.* (289) reported the identification of two neuropeptides, orexin A and orexin B, with amino acid sequences identical to Hcrt1 and Hcrt2. Orexin-producing neurons were visualized in similar sites in the hypothalamus. Intracerebroventricular injections of orexin A and orexin B stimulated feeding in a dose-related fashion with orexin A significantly more effective than orexin B, possibly due to activation of both orexin A and orexin B receptor subtypes (289). Orexin was found to be far less effective than NPY in stimulating food intake and, as with NPY and MCH (Refs. 1, 19, and 274 and our unpublished data), fasting up-regulated orexin gene expression in the hypothalamus (289). A comparative evaluation of the potency of orexins with other orexigenic signals so far examined indicates that high doses of intracerebroventricular injections of orexins than of other signals (GAL, MCH, or GABA) were needed to elicit significant stimulation of feeding (our unpublished results). Interestingly, microinjection of only orexin A in the LH, PVN, and PFH, but not in the VMN and POA, stimulated feeding (100). Thus, although orexin fibers terminate in large numbers of hypothalamic sites, the orexin receptors mediating orexigenic effects may have a limited distribution. Based on the distribution pattern of orexin receptors (290), we suggest that orexin-2R in and around LH-PVN-PFH axis may participate in stimulation of food intake (100). Horvath *et al.* (291, 292) visualized hypocretin/orexin immunoreactive fibers making direct contacts with NPY and leptin coexpressing neurons in the ARC. Further, the hypocretin neurons projecting to the ARC also appeared to be the leptin target. Consequently, it would seem that at least a part of excitatory effects of hypocretins may result from stimulation of NPY release in the PVN and surrounding neuronal sites. As seen with GAL- and POMC-producing neurons, NPY-expressing cells also established synaptic contacts with hypocretin neurons in the LH, thereby raising the likelihood of a regulatory NPY input on orexin-producing cells. These findings present additional supportive evidence to strengthen the view, as expounded in this article, that overlapping interactive appetite-stimulating pathways innervate various hypothalamic sites in the hypothalamus to participate in

coordination of the daily pattern of feeding, and NPY system is intimately involved in this process (Ref. 1 and Figs. 1 and 7).

### *G. Morphological and functional links among orexigenic signals*

The extensive anatomical and experimental evidence detailed above clearly implies and extends our hypothesis that orexigenic signals do not act one at a time, but rather an interconnected orexigenic network integrates the hypothalamic regulation of daily food intake. The connectivities of NPY neurons with other orexigenic signals, coupled with the coexpression and corelease of these signals, exemplify the operational complexities of the orexigenic network in the hypothalamus. Morphological evidence of a NPY→β-END line of communication, along with the observations that NPY stimulated β-END release and opiate receptor antagonists attenuated NPY-induced feeding, demonstrated that NPY induces feeding on its own as well as through the release of β-END.

An analogous, but more extensive, communication between NPY and GAL in several hypothalamic sites, including the ARC and PVN, exists in the hypothalamus. Intriguingly, GAL-immunoreactive fibers also synapse with a subset of β-END-immunoreactive cells, and dendrites in the ARC and the opiate receptor antagonist, NAL, inhibited feeding stimulated by GAL, thereby revealing a functional link between GAL and β-END in the daily patterning of food intake. Seemingly, an interconnected peptidergic orexigenic network of NPY→GAL→β-END represents the hard wiring of the hypothalamic circuitry regulating nocturnal feeding in the rat. Furthermore, the catecholamine NE and the amino acid GABA, coproduced with NPY and GAL, represent another mode of participation of orexigenic signals involving postsynaptic synergistic interaction. It is apparent that these interacting appetite-stimulating pathways may be employed to different degrees under various physiological circumstances and environmental challenges. That this biological redundancy is vital for energy homeostasis is evident from NPY-knockout mice. These mutant mice show a normal ingestive behavior; however, deletion of NPY in the *ob/ob* hyperphagic obese mice produced body weight closer by 40% to wild type (293, 294). This interesting observation not only underscores the importance of the role of NPY, but also raises the possibility that other interacting orexigenic signals, as demonstrated by VMN-COL model (18, 19, 63, 68), may begin to play a greater role in the daily management of energy homeostasis when NPY signaling is impaired.

## IV. Anorexigenic Signals

### *A. CRH family of peptides*

CRH was isolated and sequenced in 1981 (295). It is the primary hypothalamic hormone stimulating the release of pituitary ACTH, which stimulates corticosterone secretion from the adrenal glands. CRH-producing cells involved in regulation of the pituitary-adrenal axis are localized mainly in the pPVN. These neurons project into the external zone of the median eminence to release CRH into the hypophyseal portal system for transport to the pituitary (211, 296, 297). CRH also exerts powerful excitatory effects on arousal and locomotor activity and elicits "anxiogenic-like" effects in rats (102, 298–300). Central injection of CRH produces anorexia, as evidenced by attenuation of nocturnal and fasting-induced feeding, and diminishes feeding in a number of pharmacological and behavioral paradigms designed to evaluate ingestive behavior (13, 300, 301). These diverse biological effects of CRH are exerted at specific sites in the brain (101, 102). Indeed, multiple subpopulations of CRH-producing neurons, CRH-immunoreactive terminals, and high-affinity binding sites have been localized in various regions in the brain (302–305).

Microinjection studies revealed that the sites of anorectic action of CRH lies within the PVN, possibly mediated by CRH R1 or CRH R2 receptor types (102, 300, 306). The ability of a CRH antagonist, α-helical CRH (9–41) to attenuate the anorexic effects of CRH injection in the PVN and not in the VMH suggested a site-specific involvement of PVN CRH receptors. The report that intraventricular injection or microinjection of CRH into the PVN, and not elsewhere in the hypothalamus, inhibited NPY-induced feeding (306) further strengthened the notion that CRH, if released locally in the PVN, may tonically restrain the action of endogenous orexigenic signals.

On the other hand, several lines of evidence question the role of CRH as a physiologically relevant endogenous anorexigenic signal. There is little evidence to show that the daily pattern of CRH released locally in the PVN correlates inversely with the daily feeding pattern (211, 307). In fact, increases in CRH mRNA in the PVN, reflecting impending increased synthesis and release locally, occurred before the daily afternoon activation of the pituitary-adrenal axis, which is thought to facilitate feeding (211, 307). Further, activation of the hypothalamo-pituitary-adrenal axis is invariably observed in experimental conditions, such as fasting and food restriction, that enhance appetite and demand suppression of endogenous anorexic signals (308, 309). Adrenalectomy is known to up-regulate CRH production and release, and yet in these animals daily food intake was practically unchanged (308, 310–312). A small reduction in nocturnal feeding was attributable to glucocorticoid deficiency and the adverse impact on sympathetic nervous system activity including thermogenesis and gastrointestinal function, factors important for energy homeostasis (308–310).

Urocortin, a recently described member of the CRH family with 45% sequence homology with CRH, has been shown to be more potent than CRH in suppressing both the fasting-induced and nocturnal feeding (313, 314). Reduction of nocturnal feeding by urocortin was found to be due to a reduction in meal size and not frequency of meal bouts (314). This observation questions the physiological significance of this anorexic peptide in the nocturnal feeding marked by robust increase in both meal size and frequency. The topographies of urocortin and CRH-expressing cells in the rat brain are quite different and interesting. Urocortin-expressing cells are found in the Edinger-Westphal nucleus, the lateral superior olive, the LH and supraoptic nucleus (SON) (313, 314), but not in the PVN. The higher anorectic potency of urocortin has been attributed to a relatively higher affinity of urocortin for

CRH R2 and its splice variant CRH R2$\alpha$. Although urocortin-immunoreactive nerve fibers innervate lateral septum, VMH, and medial amygdaloid nucleus, urocortin microinjections in the VMN and not in the PVN inhibited feeding (80). Recently, Heinrichs *et al.* (315) reported that increased availability of CRH/urocortin in the hypothalamus by the chronic central infusion of rat/human $CRH_{(6-33)}$, a high-affinity CRH binding protein inhibitor, significantly decreased body weight in Zucker obese rats that normally have reduced CRH stores in the hypothalamus. Interestingly, since hyperphagia was unabated in these animals, these investigators suggested that the loss in body weight was due to CRH/urocortin-induced increase in energy expenditure and sympathetic tone produced by thermogenesis and lipolysis, a hypothesis advocated earlier by Rohner-Jeanrenaud and associates (309, 310). Thus, evidence available to date is not consistent with the notion of a direct physiological role of CRH/urocortin in the daily management of appetite.

### *B. Neurotensin*

Neurotensin (NT), isolated and characterized in the early 1970s (316, 317), inhibits spontaneous and NE-induced feeding in rats, and there is evidence that NT and dopamine act synergistically to inhibit feeding (318). The neuroanatomical mapping of NT pathways in the rat hypothalamus is consistent with the existence of anorexigenic pathways. Within the hypothalamus, NT-like immunopositive neurons exist in several distinct nuclei (319–321). Notable among these are the subset of NT-producing neurons in the ARC, PVN, and DMN. In addition, these and neighboring sites are richly innervated by NT-immunopositive fibers. Interestingly, recent studies showed that a subset of NT-positive neurons in the DMN project into both the pPVN and mPVN (83, 135), sites where microinjection of NT inhibited spontaneous feeding (322, 323). In addition, consistent with a reciprocal interaction between NPY and NT underlying hyperphagia in rodents, Wilding *et al.* (324) reported that *ob/ob* mice exhibited decreased hypothalamic NT mRNA and peptide levels in association with enhanced NPY levels and gene expression. Similarly, NT concentrations were reduced in several hypothalamic nuclei of Zucker obese (*fa/fa*) rats (325), possibly due to impaired processing of brain proneurotensin (326). These observations strongly favor a role of NT in the anorexigenic circuitry; however, experimental evidence for the physiological relevance of NT in regulation of daily pattern of feeding is lacking.

### *C. Glucagon-like peptide-1*

Glucagon-like peptide-1 (GLP-1) (7–36) amide is processed from proglucagon in intestinal L cells, and it is considered as a hormone related to the glucagon/secretin family of peptides (327, 328). Like several other gastrointestinal peptides, GLP-1 has been found in various forebrain sites and in hypothalamic sites (329, 330) that correspond with GLP-1-binding sites in the ARC and PVN (331, 332). Extensive hypothalamic innervation by GLP-1-immunoreactive fibers emanates apparently from a single population of non-catecholamine-producing neurons in the caudal portion of the nucleus of the Solitary tract (332, 333). Intraventricular administration of GLP-1 inhibited food intake in fasted rats, a response blocked by the concurrent administration of $exendin_{(9-39)}$, a GLP-1-receptor antagonist (334–336). A physiological role of GLP-1 as an anorectic or satiety factor was suggested by the observations that exendin stimulated feeding in satiated rats during the lights-on period, and daily injections of exendin augmented food intake and body weight (335). Evidence suggests that one of the sites of GLP-1 action may be the PVN where GLP-1-immunoreactive fibers terminate and where exendin blocked GLP-1-induced activation of c-FOS (335). The anorectic effects of GLP-1 may be mediated through NPY signaling because GLP-1 inhibited and $exendin_{(9-39)}$ augmented NPY-induced feeding, respectively (335, 337). Suppression of feeding by GLP-1 likely involves inhibition of postsynaptic signaling initiated by NPY in the PVN and not by suppression of NPY synthesis in the ARC. That GLP-1 may be an endogenous anorectic signal was also indicated by the report that attenuation of feeding by GLP-1 was not due to conditioned taste aversion (338, 339). Recently Goldstone *et al.* (340) reported coexpression of leptin receptor and GLP-1 mRNA in BS neurons, and the GLP-1 receptor antagonist, $exendin_{(9-39)}$, blocked the leptin-induced inhibition of food intake and body weight, thereby suggesting that the GLP-1 pathway may be one of the mediators of the anorectic effects of leptin. In this context, it is important to note that GLP1-R knockout mice did not exhibit any abnormalities in feeding behavior (341).

### *D. Melanocortin and agouti protein*

Another fascinating molecular underpinning of the hypothalamic appetite-regulatory system is exemplified by the obesity syndrome in agouti lethal ($A^Y$/a) mice (342, 343). In this mutant mouse, disruption in intrahypothalamic anorexigenic signaling is caused by the agouti protein acting through MC4 receptors. This causes maturity-onset obesity, hyperinsulinemia, hyperglycemia, and significant increase in linear growth accompanying fat cell hypertrophy, a phenotype akin to most human forms of obesity (342–346). The melanocortin, $\alpha$MSH, a nonopioid peptide encoded by the POMC gene, is distributed throughout the hypothalamus (347, 348) and, unlike $\beta$-END and other opiates that stimulated ingestive behavior through activation of $\mu$ and $\kappa$ receptors, $\alpha$MSH inhibited food intake. Although not yet certain, it remains possible that $\alpha$MSH-induced inhibition of feeding is mediated through MC4-R. Of the five melanocortin receptors cloned, only two related receptor subtypes, MC3-R and MC4-R, are primarily expressed in the brain (349–354). MC4-R mRNA-expressing neurons are found in more than 100 sites in the brain including several within the hypothalamus, such as the PVN, VMH, DMN, and nuclei that occupy the medial zone of the hypothalamus (355, 356).

Huszar *et al.* (346) reported that a targeted mutation of the MC4-R caused maturity-onset obesity, with symptoms of hyperphagia, hyperinsulinemia, and hyperglycemia, remarkably similar to those associated with the agouti obesity syndrome (342, 344, 346). Neural synapses relaying information through the $\alpha$MSH-MC4-R pathway seem to be in-

timately involved in regulating energy balance. Involvement of hypothalamic MC4-R in feeding is supported by the observations that intracerebral administration of a potent MC4-R agonist inhibited feeding in hyperphagic or NPY-injected mice and in mice with a dominant mutation at the agouti locus ($A^Y$), and administration of a specific melanocortin antagonist (SHU9119) counteracted the inhibitory effects of MC4-R agonists (346). It should be noted that injections of SHU9119 potentiated nocturnal intake but were completely ineffective in eliciting feeding in satiated rats during the daytime. In a series of recent investigations with rather more selective MC4-R antagonists (*e.g.*, HS104 and HS024), Schiöth and co-workers (357–361) demonstrated that these antagonists augmented feeding in satiated rats during the daytime, and long-term intraventricular infusion increased food intake and body weight gain leading to obesity. Taken together, these findings suggest a tonic restraint on feeding by melanocortin, possibly dependent on αMSH and mediated by MC4-R. Removal of this restraint, as seen in lethal yellow ($A^y$/a) mice by the agouti protein, in MC4-R knockout, or after MC4-R antagonists, leads to hyperphagia and obesity.

Although Kesterson *et al.* (97) failed to observe it in several obesity models, including lethal yellow ($A^Y$/a), MC4-R knockout, and *ob/ob* mice, POMC mRNA expression has been shown to be reduced either in the rostral part or throughout the ARC of *ob/ob* and *db/db* obese mice (362–364). Leptin treatment of leptin-deficient *ob/ob*, and not leptin-resistant *db/db* mice, normalized POMC mRNA expression. Similarly, fasting-induced diminution in POMC mRNA in the ARC was prevented by leptin treatment in rats (362–364). Based on these observations, together with the fact that POMC neurons express leptin receptors (362), it is reasonable to formulate the hypothesis that POMC neurons exert a tonic restraint on feeding through αMSH-MC4-R signaling in rodents and that a diminution or defect in the leptin-POMC-αMSH-MC4-R signaling axis may, in part, be responsible for hyperphagia in genetically obese models and for the increased drive for food in energy-restricted rodents (362–365). However, as alluded to earlier, the fact that POMC neurons also produce orexigenic β-END, this singular role of αMSH-MC4-R signaling within the ARN remains unproven. Further, Boston *et al.* (366) recently showed that weight gain and hyperphagic effects of defective POMC signaling and leptin deficiency may not be linked. Indeed, double-mutant lethal yellow ($A^Y$/a) leptin-deficient (*ob/ob*) mice displayed independent and additive effects on weight gain and, furthermore, resistance to leptin in this model was related to desensitization of leptin signaling.

More recently, a novel gene, ART, with high homology to agouti gene encoding agouti-related protein (AgrP), has drawn considerable attention because of its localization in several hypothalamic sites, including in ARC where neurons coexpressing NPY and ART/AgrP have been visualized (39, 40). Like NPY mRNA expression, ART was expressed in markedly higher amounts in the hypothalami of obese (*ob/ob*) and diabetic (*db/db*) mice, a response found to be independent of adrenal glucocorticoids (54, 55). Broberger *et al.* (367) reported that NPY- and AgrP-expessing cells in the ARC project extensively to various hypothalamic and extrahypothalamic regions in the brain, thereby suggesting a much broader area involved in feeding stimulated by NPY. AgrP is a potent, selective antagonist of MC3-R and MC4-R receptor subtypes, the melanocortin receptors implicated in control of energy balance (54, 55). Since NPY-producing neurons in the ARC project to the PVN to release NPY for stimulation of feeding, these findings raised the possibility that NPY and AgrP are coreleased in the PVN from the nerve terminals of ARC NPY- and ART mRNA-coexpressing neurons. One function of AgrP released in the PVN would be to curtail the restraints on the inhibitory effect of αMSH.

On the other hand, in keeping with the notion that the drive for food is chemically coded through the release of orexigenic signals (1), a large body of morphological and experimental evidence points to NPYergic signaling for curtailing the restraint by POMC α-melanocortin receptors. NPY-producing neurons are synaptically linked with ARC POMC neurons (34) that express $Y_1$ receptors (166, 167), and NPY administration promoted the release of the orexigenic β-END (56), inhibited the release of the anorexigenic αMSH (285), and suppressed POMC gene expression in the ARC (268). Also, MC4-R antagonist-induced feeding appeared to be mediated by NPY $Y_1$ receptors (361). Thus, as proposed earlier (1, 12), increased NPYergic signaling in genetically obese rodent models, and in response to energy deficiency in food-deprived and food-restricted rats and normal nighttime feeding (171), is likely responsible for curtailing the restraint by melanocortin signaling.

These observations, when taken together with the fact that NPY-producing neurons in the ARC coexpress GABA and NPY, and GABA can coact to amplify feeding (36–38), it becomes obvious that ARC contains a unique subpopulation of neurons that coproduce NPY, the most potent orexigenic signal known, an amino acid, GABA, and a neuropeptide, AgrP; both of the coexisting signals seemingly enhance, by disparate mechanisms, the effectiveness of NPY.

Thus, we propose that a three-prong interplay, initially involving increased NPYergic signaling on it own, and then through activation of orexigenic β-END, αMSH, and ART, results in nocturnal feeding in rodents. Evidence of increased NPYergic signaling, not only within the ARC but also in the DMN in genetic and experimental models of obesity, underscores the role of NPY as a primary neural factor in evoking hyperphagia. Indeed, suppression of NPYergic signaling by leptin, involving either a direct action or after entering the brain, on NPY neurons in the ARC as in leptin-deficient *ob/ob* mice (224, 368, 369), or by CNTF in leptin-deficient fasted and food-restricted rats (218, 219), leptin-deficient *ob/ob* mice (370), and leptin-resistant *db/db* mice, resulted in markedly suppressed appetite. Undoubtedly, the NPY system, on its own and through multiple channels of communication with components of the orexigenic and anorexigenic networks, stimulates appetite. Defective POMC signaling through the MC4-R amplifies activation of feeding by NPY and possibly other orexigenic signals.

### *E. Cocaine and amphetamine-regulated transcript*

Recently, a new neuropeptide, CART (cocaine and amphetamine-regulated transcript), was localized in the rat

brain and shown to be distributed in feeding-related sites in the hypothalamus (57–59). Intraventricular administration of CART inhibited nocturnal as well as fasting-induced feeding in mice and rats (60). Extensive series of investigations in two laboratories presented strong evidence to show that CART may be a physiologically relevant anorectic signal (60, 61). This view is supported by the following evidence: 1) Administration of antiserum against CART, to neutralize the anorectic impact, increased nighttime feeding. 2) CART mRNA was localized in the ARC, PVN, SON, rostral part of the VMH, anterior paraventricular nucleus of the hypothalamus, and several other nuclei in the diencephalon. Fiber projections from these CART-producing cells were located in a large number of hypothalamic nuclei. 3) Expression of CART mRNA in response to fasting decreased in the ARC and DMH in normal rats. 4) In the genetically obese rodents, Zucker (*fa/fa*) rats and *ob/ob* mice, CART gene expression in the ARC and DMH was decreased relative to wild-type controls. 5) Leptin administration to *ob/ob* mice, which lack leptin, increased CART mRNA in the ARC to the range seen in wild-type lean control mice. This response was associated with decrease in food intake in the *ob/ob* mice. 6) Interestingly, CART administration markedly inhibited the NPY-induced feeding in fasted and normal rats. Although these results suggested an inhibitory action of CART exerted at postsynaptic levels, immunocytochemical studies showed a close apposition of NPY-containing terminal with the perikarya of CART peptide-immunoreactive neurons in the pPVN, possibly representing NPY→CART communication line. This observation is highly suggestive of a regulatory role of NPY on CART output, in a fashion similar to NPY→GAL, NPY→POMC, NPY→orexin signaling. Thus, CART-containing neurons appear to represent one of the most powerful physiological anorexic signaling pathways.

## V. Leptin

### A. An adipocyte signal

The concept that appetite-restraining signal(s) from adipocytes are integral components of the feedback loop between the periphery and the brain for energy homeostasis gained firm ground with the cloning of the obesity (*ob*) gene from adipocytes (16, 17). Leptin, the *ob* gene product, is a secreted protein relaying a satiety signal to the hypothalamus after entering the brain by an active saturable system (17, 371). Administration of leptin either peripherally or centrally to genetically obese mice (*ob/ob*), and their lean counterparts, reduced food intake and body weight (17, 228, 229). In general, relatively high doses were needed to elicit a significant impact in laboratory rodents. Although a few reports have indicated rapid suppression of spontaneous nighttime feeding by leptin administered before lights off (372), there is a consensus that suppression of food intake is not immediate. The observation of 8–10 h of latency of response to leptin (218, 373) is of considerable importance in further elucidating the role of leptin and leptin receptors in nocturnal feeding (Fig. 2) and the daily management of energy homeostasis in rodents. This issue gains considerable significance when the circulating pattern of leptin is correlated with the feeding behavior of rats either feeding freely or maintained on a restricted regimen (Fig. 2; Refs. 120, 121, and 123). Apparently, leptin does not inhibit spontaneous episodic feeding, but reduces the amount of intake in a feeding episode (373). Chronic high levels of leptin, achieved by intravenous or intraperitoneal injections of a recombinant adenovirus expressing the rat leptin cDNA, resulted in a marked reduction in food intake and body weight loss in genetically obese mice and normal rats (374, 375). Similarly, continuous subcutaneous infusion of leptin in the physiological range decreased body weight and food intake in lean and several genetic models of obese mice; chronic infusion of low doses into the third cerebroventricle replicated the anorectic and weight-reducing effects of subcutaneously infused leptin (376).

### B. Site of action

Although several lines of evidence suggest that the hypothalamus is the primary brain site targeted by leptin (17, 228, 229, 372), the precise site within the hypothalamus cannot be ascertained at present. *In situ* hybridization studies showed that the biologically active, long form of the leptin receptor (OB-Rb) is produced in various extrahypothalamic and hypothalamic sites in the mouse brain. Strong expression of OB-Rb mRNA was seen throughout the ARC, VMH, PVN, LH, and ventral premamillary nucleus ($PM_V$; Ref. 368). On the other hand, Schwartz *et al.* (105) visualized OB-Rb in the rat brain in limited sites with a strong signal in the ARC and weaker expression in the VMN and DMN and, surprisingly, no expression in the PVN. Whether these differences in expression of OB-Rb mRNA in rats and mice represent species differences remains to be determined.

Microinjection of leptin into the ARC, VMH, and LH inhibited 24-h food intake in rats, with the ARC being the most sensitive site (78, 79). Several laboratories have attempted to identify the hypothalamic sites of leptin action by evaluating FOS-like immunoreactivity (FLI) as a marker of neuronal activation. Woods and Stock (377) observed FLI selectively in the PVN of ob/ob but not lean mice at 3 h after a systemic injection of leptin. On the other hand, Elmquist *et al.* (88) reported a wider distribution of FLI in various hypothalamic and extrahypothalamic sites in response to peripheral injection of leptin in rats. In particular, FLI was visualized in the dorsomedial part of the VMH, the posterior part of the DMN and $PM_V$. Additionally, FLI was seen sparingly in the pPVN, but remarkably, not in the ARC. In contrast, central administration of leptin elicited FLI in the PVN and DMN only (378). Another regional difference in FLI expression observed by these two laboratories was in the medial pPVN where peripheral leptin injections readily evoked FLI, but central leptin injection was ineffective (88, 378). Our studies showed that central injection of leptin stimulated FLI expression in the PVN, DMN, and $PM_V$ and little expression in the ARC (89, 90). This lack of FLI in the ARC in response to leptin is intriguing and requires further investigation.

A comparative analysis of OB-Rb mRNA localization by *in situ* hybridization with those of neuronal FLI activation in response to leptin microinjection showed considerable regional overlap within the hypothalamus, allowing one to

infer that the putative sites of leptin action correspond closely to the broad neuroanatomical substrate concerned with the control of energy homeostasis (Fig. 1; see *Sections III* and *IV*). The list of leptin target neuronal systems has recently grown to include subpopulations of orexigenic and anorexigenic signal-producing neurons, thereby raising the possibility of a complex neuronal substrate for leptin feedback involving diminution or restraint in the output of orexigenic signals concurrent with activation of anorexigenic signals (see below).

*1. Orexigenic signal-producing network.*

*a. NPY signaling:*

*i) Leptin and NPY synthesis and release.* Perhaps the most revealing finding of the adipocyte-hypothalamic signaling pathway is that a subpopulation of NPY-producing cells in the hypothalamus may be one of the targets of leptin action. The observations that 1) OB-Rb receptor and NPY are coexpressed in the ARC (369, 379–381); 2) leptin inhibited NPY gene expression in the ARC of genetically obese (*ob/ob*) mice and in normal and food-deprived rats (224, 372); and 3) reduced NPY levels in the ARC, DMN, and PVN (372), suggested that leptin may regulate the availability of NPY for release. Leptin decreased NPY release *in vitro* from hypothalamic fragments of *ob/ob* mice perifused with corticosterone, but not in the absence of corticosterone (224), and KCl-induced NPY outflow from the hypothalamus was attenuated by leptin (382). Although these findings suggested that leptin may rapidly decrease NPY release from the corticosteroid-primed whole hypothalami of leptin-deficient mice, similar rapid action of leptin on NPY release has not been replicated in rats. There was no effect of leptin on NPY release from the microdissected ME-ARC, VMH, or PVN of normal or food-deprived rats (S. P. Kalra, unpublished), and NPY efflux in perfusates collected from PVN were not affected by leptin (383). It is possible that NPY release may not be the primary locus of action; instead, leptin may restrain feeding by restricting the availability of NPY on a long-term basis and by counteracting the orexigenic effects of NPY at postsynaptic target sites (see below).

*ii) Leptin and NPY-induced feeding.* Leptin suppressed NPY-induced feeding acutely and on a long-term basis (219). Daily injection of leptin decreased food intake and body weight gain concomitant with not only diminished NPY gene expression in the ARC but also by interfering with NPY action (219). In long-term leptin-treated rats, stimulation of feeding in response to intraventricular NPY was nearly abolished (Fig. 6). Even in the *ob/ob* mice with little endogenous leptin, Smith *et al.* (384) reported a dose-dependent decrease in NPY-induced feeding by exogenous leptin. Leptin has also been reported to suppress GAL- and MCH-induced feeding (385).

Analysis of c-FOS to identify the site(s) of inhibition of NPY-induced feeding by leptin showed a narrow field of interaction (89, 90). Although NPY stimulated FLI in several hypothalamic sites, mPVN was the only region in which leptin preinjection significantly decreased FLI in association with reduced food intake (Fig. 3; Refs. 89 and 90). A similar diminution in mPVN FLI was noted when NPY-induced feeding was inhibited by the NPY $Y_1$ receptor antagonist,

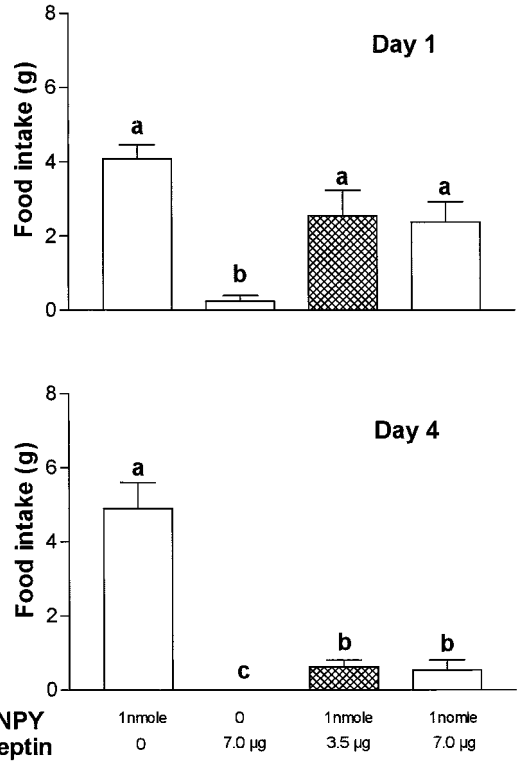


FIG. 6. Suppression of NPY-induced food intake by a daily injection of leptin intraventricularly for 4 days. *Dissimilar superscripts* represent statistically significant differences from other treatment groups ($P < 0.05$). [Reproduced with permission from B. Xu *et al.: Endocrinology* 139:466–473, 1998 (219) © The Endocrine Society].

1229U91 (164). Evidently, a subpopulation of neurons selectively in the mPVN is the target of NPY and leptin interaction (Fig. 3). In addition, since leptin and NPY together increased FLI in the DMN, it is likely that diminution in FLI expression by leptin in the mPVN may be secondary to the synergistic activation of DMN neurons by leptin and NPY (90, 164). Thus, the participation of the DMN-mPVN axis in the restraining effects of leptin on NPY-induced feeding sets the stage for further exploration of these two hypothalamic nuclei.

*b. Other orexigenic signals:* Håkansson *et al.* (379) recently localized the OB receptor (OB-Rb) with an antibody that recognized all isoforms of this receptor on the periphery of NPY- and POMC-producing neurons in the ARC and MCH-producing neurons in the LH. Coexpression of OB-Rb with POMC mRNAs, and with GAL mRNA in the ARC, also has been reported (362, 363). Orexin-producing neurons in the LH also express OB-Rb (291, 292). Taken together, these findings raise the possibility that POMC-, MCH-, GAL-, and orexin-containing neurons may be the targets of leptin. Leptin suppressed feeding evoked by MCH and GAL. Like NPY, whether the simultaneous diminution in the secretion of these orexigenic peptides underlies reduction of food intake and body weight in the daily management of energy balance remains to be demonstrated.

*2. Anorexigenic signal-producing network.* Localization of OB-R immunoreactivity or OB-Rb mRNA showed that the leptin receptor is coexpressed in neurons producing the anorexi-

genic peptides, ACTH-positive POMC neurons in the ARC (362, 363), and GLP-1-producing neurons in the nucleus of the solitary tract (340, 386). Although inconsistent with a recent report (387), leptin injections to leptin-deficient *ob/ob* mice suppressed POMC mRNA (363). The decrease in the ARC POMC mRNA due to fasting was also prevented by leptin treatment. These results have been interpreted to mean that increased POMC mRNA in leptin-treated rats represents increased release of αMSH in the PVN which, in turn, decreases feeding. However, this is uncertain because of the possibility that increased POMC mRNA also reflects increased release of the orexigenic opioid peptides (56). On the other hand, the ability of leptin to inhibit food intake was attenuated by blockade of MC4 receptors, suggesting a role of αMSH-MC4-R in mediating inhibition of food intake (365), a finding not in line with those of Boston *et al.* (366). Interestingly, the GLP-1 receptor antagonist exendin blocked leptin's effects and because GLP-1 producing neurons in the nucleus of the solitary tract express OB-Rb, it is also possible that GLP-1 neurons are a potential target for leptin (340, 386). Consequently, whether leptin suppressed feeding primarily by diminishing the release and/or action of orexigenic signals alone or together with augmenting the signaling of anorexigenic peptides remains to be clarified.

### *C. Is leptin a physiological satiety signal?*

*1. Leptin synthesis and secretion in relation to feeding behavior.*

*a. Correlation with ingestive behavior in rodents:* As summarized in the preceding section, overwhelming evidence demonstrates that exogenous leptin inhibits food intake in rodents. A few exceptions are rats with either lesions in the VMH (145, 146) or disruption of neural signaling by neurotoxins in the VMN (18, 19, 63, 388) and the genetically obese rats (fa/fa Zucker) and mice (*db/db* and $A^Y$/a Agouti yellow; 16, 227–229). These rodents are obese, hypersecrete leptin, and are resistant to the central antiappetite action of leptin.

The precise role of leptin in the daily patterning of ingestive behavior is not yet clear. The assumption that leptin restrains food intake to maintain body weight within the narrow set point for an individual is not supported by current data on the daily patterns of adipocyte leptin gene expression and circulating levels. Rodents are a nocturnal species consuming almost 80–90% of their daily intake during the lights-off period in conjunction with increased general activity. There is little, if any, intake before the onset of the dark phase. Arousal and increased general activity normally precedes ingestive behavior after onset of the dark phase. During the lights-on period, general activity is minimal with rats displaying a few short-lived feeding episodes (389). Saladin *et al.* (390) reported that leptin mRNA levels in adipose tissue of mice showed a daily rhythm with lowest levels during the entire lights-on phase, but it increased during the dark phase, beginning at 2000 h and rapidly peaking at 0400 h. On the other hand, the daily pattern of serum leptin levels was different and lagged considerably behind the peaks and valleys in leptin mRNA levels (390). Serum leptin levels in mice were lowest at 2 h into the dark phase (1800 h, lights on 0400–1600 h), followed by a rise beginning at 2400 h and peaking by 0400 h. A slow rate of decrease in circulating leptin levels occurred throughout the lights-on phase to reach nadir at 2 h into the dark phase. In our studies (120, 121, 123), leptin mRNA in rat adipocytes increased abruptly before the onset of feeding at the time of lights off (1900 h; Fig. 2). Thereafter, during the intense period of feeding, leptin mRNA levels increased to peak at 2300 h and then declined along with the cessation of nighttime food consumption. This daily pattern in the steady state levels of adipocyte leptin mRNA and leptin-R is apparently characterized by enhanced gene expression before onset of the dark phase and ingestive behavior. This observation is strongly suggestive of the existence of an independent mechanism regulating OB gene expression that is not driven by lights-off signal. Surprisingly, on the other hand, a markedly different temporal pattern in serum leptin levels also exists in these rats. Leptin secretion increased after lights off to peak at 2300 h and then fell progressively during the late dark-phase concomitant with ongoing feeding. Leptin secretion thereafter decreased to a nadir at 1100 h during the light phase, when rats display little feeding behavior.

This dichotomy in the temporal patterns of leptin gene expression and circulating leptin levels in mice and rats provides new interpretations for the complex role of leptin in regulating daily ingestive behavior and maintenance of body weight within the set point of the individual. Evidently, neither the onset of feeding nor the lights-off signal is the trigger for increased gene expression in adipocytes. Instead, we propose that a distinct neural mechanism, which occurs in anticipation of the animal's need to increase leptin secretion during the nocturnal feeding, initiates this antecedent increase in leptin gene expression. However, maintenance of nocturnal high levels of leptin gene expression and secretion are coupled to food intake because overnight fasting abolished the late dark-phase increments in leptin gene expression (213, 391), and restricting the availability of food to lights-on phase accordingly moved increased leptin secretion into the interval of robust feeding (120, 121, 123).

Since blood leptin levels are basal during the lights-on phase, contemporaneous with little evidence of feeding, it is reasonable to assume that the feeding-associated rise in leptin secretion constitutes the feedback signal to the hypothalamus, to first diminish and eventually terminate the urge to eat. This impact is sustained during the lights-on period interspersed with very few brief feeding episodes. Thereafter, as increased availability of Ob-Rb imparts enhanced sensitivity to leptin, as reflected by augmented hypothalamic Ob-Rb gene expression, the feeding-associated leptin rise may greatly assist in reducing appetite (Fig. 2). Overexpression of hypothalamic Ob-Rb mRNA, associated with reduced leptin levels and increased sensitivity toward leptin action, although not consistently observed (392, 393), is in line with the interplay between antecedent increased leptin sensitivity and prandial rise in circulating leptin as responsible for exerting a longlasting suppression of feeding seen during the lights-on phase.

*b. Correlation with ingestive behavior in humans:* It is well documented that Ob mRNA levels in adipocytes and serum leptin levels correlate with body fat mass and weight in normal and obese patients (220, 227, 394–397). Fasting rapidly decreased secretion and, conversely, overfeeding for

short or long periods augmented leptin secretion parallel with the attendant increases in percentage of body fat (394–398). In humans, there is a highly organized pattern of leptin secretion over a 24-h period. In general, the circadian pattern is characterized by basal levels between 0800–1200 h, rising progressively to peak between 2400–0400 h, and receding steadily to a nadir by 1200 h (400). Unlike that in rodents, increases in leptin secretion do not appear to be driven by meal patterns. Leptin is secreted in a regular pulsatile fashion with an interpeak interval of about 44 min (400, 401), and the circadian rhythm is attributable solely to increased pulse height. Interestingly, the circadian pattern of leptin secretion is preserved in obese patients, and elevated blood levels could be accounted for by increased leptin pulse height. In women with anorexia nervosa, leptin levels are maintained in the low range (402). These circadian and pulsatile patterns of fluctuations in blood leptin levels imply that neural and neurohumoral components in the brain may regulate leptin secretion from adipocytes. Because of the rapid development of leptin resistance in humans, our new challenge is to elucidate the mechanism by which these two patterns of leptin secretion regulate appetite and body weight gain in humans.

### *D. Leptin signal transduction and leptin resistance*

A limited number of studies examined the electrophysiological events triggered by leptin in target cells in the ARC and VMH. Leptin hyperpolarized glucose-responding hypothalamic cells by activation of potassium currents in lean, but not Zucker (*fa/fa*), rats (403). In rat brain slice preparations, leptin rapidly altered synaptic transmission in ARC neurons by attenuating evoked excitatory action potentials (404). Localization of Ob-Rb protein in the hypothalamus and extrahypothalamic sites revealed another intriguing finding (405). Light microscopic analysis showed Ob-Rb immunolabeling associated with the Golgi apparatus of the neurons and glial cells in the ARC, VMN, and pPVN and mPVN (405). Ob-Rb immunolabeling was selectively confined to *cis*, medial, and *trans* cisternae and absent in vesicles in close proximity to either faces of the stack. These novel observations are suggestive of a role for leptin in modulating intracellular events, possibly in processing of neurotransmitter/neuromodulators, the functional property of the Golgi apparatus.

Further, the insight that Ob-Rb has homology to members of class I cytokine receptor family, including those of the potent anorectic CNTF (173, 218, 219) sparked investigation to explore similarities in molecular events in intracellular signal transduction engaged by cytokines and leptin (406). The current evidence indicated that leptin elicited a dose-dependent activation of the transcription factor STAT3 (signal transducer and activator of transcription 3) in the hypothalamus of mice and rats (407–409). This activation is suspected to augment c-*fos* expression, ultimately culminating in modulation of orexigenic and anorexigenic output. The identity of tyrosine kinases (Janus Kinases, JAKS) and other upstream cascades subsequent to binding of leptin to Ob-Rb remains to be ascertained (409, 410).

Leptin insensitivity in many rodent models of obesity is apparently due to structural aberrations in Ob-Rb itself or defective transport of leptin across the blood-brain barrier. There is also a strong possibility that leptin resistance may result from defects located downstream in the signal transduction pathway. Soon after the cloning of a family of cytokine-inducible inhibitors of signaling, the SOCS (suppressor-of-cytokine signaling; Ref. 411), Bjorback *et al.* (412) reported that leptin activated the expression of SOC-3 in the hypothalamic areas corresponding to Ob-Rb expressing cells. Further, forced expression of SOC-3 blocked leptin-induced signal transduction in a mammalian cell line and, interestingly, basal SOC-3 mRNA in the ARC and DMN were elevated in leptin resistance model, lethal yellow ($A^y/a$) mice. The fact that SOC-3 is a leptin-inducible negative intracellular regulator of leptin signal transduction, one can envision that up-regulation of SOCS contributes toward development of leptin resistance in several rodent models and obese individuals.

## VI. Summary

Various aspects of the complex spatio-temporal patterning of hypothalamic signaling that leads to the development of synchronized nocturnal feeding in the rat are critically examined. Undoubtedly, as depicted in Fig. 7, a distinct ARN in the hypothalamus is involved in the control of nocturnal appetite. At least four basic elements operate within this ARN. These are: 1) A discrete appetite-driving or orexigenic network of NPY, NE, GABA, GAL, EOP, and orexin transduces and releases appetite-stimulating signals. 2) Similarly, anorexigenic signal-producing pathways (*e.g.*, CRH, GLP-1, $\alpha$MSH, and CART) orchestrate neural events for dissipation of appetite and to terminate feeding, possibly by interrupting NPY efflux and action at a postsynaptic level within the hypothalamus. It is possible that some of these may represent the physiologically relevant "off" switches under the influence of GABA alone, or AgrP alone, or in combination with NPY released from the NPY-, GABA-, and AgrP-coproducing neurons. 3) Recent evidence shows that neural elements in the VMN-DMN complex tonically restrain the orexigenic signals during the intermeal interval; the restraint is greatly aided by leptin's action via diminution of orexigenic (NPY) and augmentation of anorexigenic (GLP-1, $\alpha$MSH, and CART) signals. Since interruption of neurotransmission in the VMN resulted in hyperphagia and development of leptin resistance, it seems likely that the VMN is an effector site for the restraint exercised by leptin. The daily rhythms in leptin synthesis and release are temporally dissociable because the onset of daily rise in leptin gene expression in adipocytes precedes that in leptin secretion. Nevertheless, these rhythms are in phase with daily ingestive behavior because the peak in circulating leptin levels occurs during the middle of the feeding period. These observations, coupled with the fact that circulating levels of leptin are directly related to adiposity, pose a new challenge for elucidating the precise role of leptin in daily patterning of feeding in the rat. 4) A neural timing mechanism also operates upstream from the ARN in the daily management of energy homeostasis. Although the precise anatomical boundaries are not clearly defined, this device is likely to be composed of a group of neurons that integrate incoming internal and external information for the

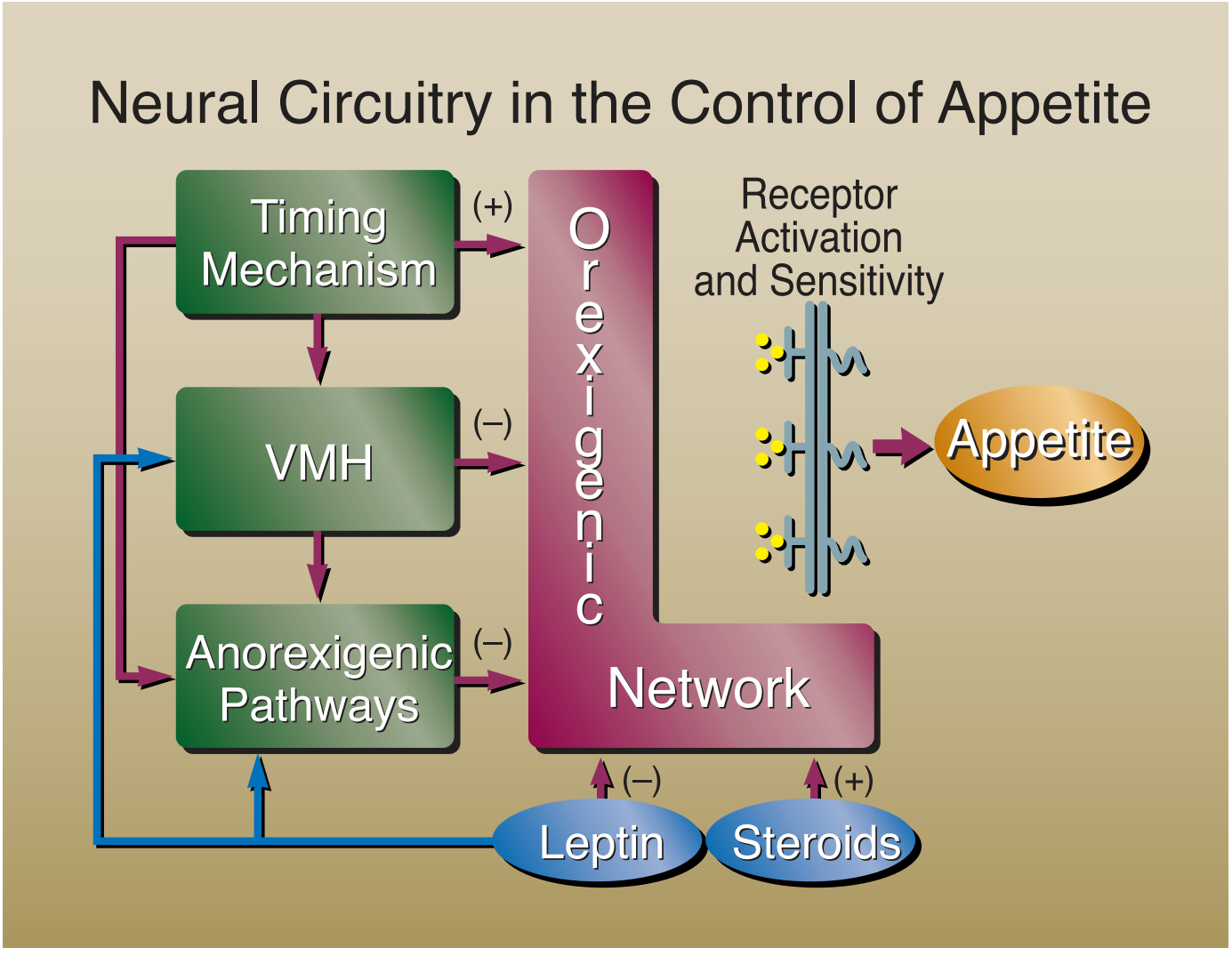


FIG. 7. A schematic representation of the four elements of the ARN in the hypothalamus. The two basic elements of the circuitry are the orexigenic and anorexigenic signal-producing networks. Extensive evidence shows that various orexigenic producing neurons are morphologically and functionally linked to provide overlapping pathways to evoke feeding behavior. The neurosecretory function of these is regulated by information from the brain-timing mechanism, which in a timely fashion either directly, or indirectly through multisynaptic routes possibly traversing the VMH, curtail the restraint of anorexigenic signals. This results in the release of appetite-stimulating signals and feeding. Peripheral hormonal signals such as adipocyte leptin, adrenal glucocorticoids, and gonadal steroids also modulate the output of orexigenic and anorexigenic signals. Coordinated operation of these components of the appetite-regulatory circuitry maintains the daily pattern of food intake. A newly identified feature of this circuitry is the dynamics of receptor activation and sensitivity as directed by the interaction between peripheral hormones and the orexigenic and anorexigenic signals. An analysis of the neurochemical etiology of various experimental models of hyperphagia reveals that both overabundance and low abundance of orexigenic signals (*e.g.*, NPY) at the receptor level cause unregulated eating behavior leading to hyperphagia. For details see text. (+), Stimulatory; (−), inhibitory; *arrows* depict the direction of information flow. [Adapted with permission from S. P. Kalra: *Neuron* 19:227–230, 1997 (1) © Cell Press].

timely onset of the drive to eat. Evidently, this network operates independently in primates, but it is entrained to the circadian time keeper in the SCN of rodents. Apart from its role in the onset of drive to eat, the circadian patterns of gene expression of NPY, GAL, and POMC denote independent control of the timing device on the synthesis and availability for release of orexigenic signals. The VMN-DMN-PVN complex is apparently an integrated constituent of the timing mechanism in this context, because lesions in each of these sites result in loss of regulated feeding.

The accumulated evidence points to the PVN and surrounding neural sites within this framework as the primary sites of release and action of various orexigenic and anorexigenic signals. A novel finding is the identification of the interconnected wiring of the DMN-mPVN axis that may mediate leptin restraint on NPY-induced feeding. The chemical phenotypes of leptin and NPY target neurons in this axis remain to be identified.

These multiple orexigenic and anorexigenic pathways in the hypothalamic ARN appear to represent redundancy, a characteristic of regulated biological systems to provide a "fail-safe" neural mechanism to meet an organism's constant energy needs for growth and maintenance. Within this formulation, the coexisting orexigenic signals (NPY, NE, GAL,

GABA, and AgrP) represent either another level of redundancy or it is possible that these signals operate within the ARN as reinforcing agents to varying degrees under different circumstances.

A most revealing outcome to explain the pathophysiology of unregulated feeding and hyperphagia emanated from the careful analysis of the relationship between NPY synthesis and release and food intake. Both overabundance and low abundance of NPY in the ARC-PVN axis appear to lead to unregulated phagia and attendant obesity. Overabundance of NPY, as evident in genetically obese and diabetic rodents, stimulated hyperphagia by increased postsynaptic receptor stimulation. On the other hand, in conditions of marked diminution of NPY availability at target sites, as seen in rats with disrupted neural signalings in the VMH, postsynaptic receptor supersensitivity and increased abundance of NPY receptors exaggerate the drive for food. Diminution in NPY production in the ARC experimentally by disruptions in VMN signaling resulted in up-regulation of NPY in novel sites, concomitant with up-regulation of GAL and increased responsiveness to GAL. Similar responses in NPY and GAL signaling were seen in genetic models of obesity. Thus, one can envision that a loss of the tight control on NPY neurosecretion resulting either in overabundance or low abundance evoked hyperphagia and attendant increase in body weight.

Detailed examination of the disturbances in neural and molecular sequelae in genetically obese rodents showed that impaired anorexigenic signaling also produced unregulated feeding and abnormal body weight gain. This is best exemplified by two rodent models, one displaying leptin and the other MCR-4 receptor deficiency in the hypothalamus. Deficiency in CART production in *ob/ob* and Zucker rats is another example. Thus, an imbalance in the operation of either orexigenic or anorexigenic pathways in the ARN (Fig. 7) perturbs the regulatory microenvironment leading to hyperphagia. Among anorexigenic signals, cytokines are the only molecules identified to date that produce anorexia and severe loss of body weight; lack of leptin, but not other members of the cytokine family, leads to hyperphagia.

Evidence to date from our laboratory clearly showed that impaired signaling within the VMN is responsible for imparting leptin resistance. Ongoing intensive research to identify sequalae in signal transduction downstream from Ob-Rb activation is likely to reveal new intracellular molecules engaged in sustaining leptin insensitivity.

This expanding knowledge of the hypothalamic ARN has provided new insights, at several levels, for designing therapeutic strategies to control appetite and body weight (Fig. 7). It is apparent that the efficacy of antiappetite drugs will depend both on their effectiveness to curtail the availability of orexigenic and/or enhance the availability of anorexigenic signals at target sites while preventing the development of receptor supersensitivity (Ref. 1 and Fig. 5). Future research should focus on characterizing the precise morphological links among components of the appetite-regulating circuitry and on elucidating the cellular and molecular sequelae associated with the orderly progression of information leading to, during, and after synchronized feeding episodes. Such insight is likely to shed light on various loci in the circuitry that may be disrupted by inappropriate environmental and hormonal events underlying the pathophysiology of hyperphagia and abnormal body weight gain.

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## References

1. **Kalra SP** 1997 Appetite and body weight regulation: is it all in the brain? Neuron 19:227–230
2. **Stunkard AJ** 1996 Current views on obesity. Am J Med 100:230–236
3. **Bray GA, Fisher J, York DA** 1990 Neuroendocrine control of the development of obesity: understanding gained from studies of experimental animals. Front Neuroendocrinol 11:128–181
4. **Wadden TA** 1993 The treatment of obesity: an overview. In: Stunkard AJ, Wadden TA (eds) Obesity Theory and Therapy. Raven Press, New York, pp. 197–217
5. **Perri MG, Nezu AM** 1993 Preventing relapse following treatment of obesity. In: Stunkard AJ, Wadden TA (eds) Obesity Theory and Therapy. Raven Press, New York, pp. 287–299
6. **Bray GA** 1996 Mechanism for development of genetic, hypothalamic and dietary obesity. In: Bray GA, Ryan DH (eds) Molecular and Genetic Aspects of Obesity. LSU Press, Baton Rouge, LA, pp. 2–66
7. **Stewart-Agras W, Kraemer HC** 1984 The treatment of *anorexia nervosa*: do different treatments have different outcomes? In: Stunkard AJ, Stellar E (eds) Eating and Its Disorders. Association for Research in Nervous System and Mental Disease. Raven Press, New York, vol. 62:193–207
8. **Crisp AH** 1984 The psychopathology of *anorexia nervosa*: getting the heat out of the system. In: Stunkard AJ, Stellar E (eds) Eating and its Disorders. Association for Research in Nervous System and Mental Disease. Raven Press, New York, vol. 62:209–234
9. **Moldawer LL, Lowry SF, Ceranii A** 1988 Cachectin: its impact on metabolism and nutritional status. Annu Rev Nutr 8:585–609
10. **Plata-Salamań CR** 1991 Immunoregulators in the nervous system. Neurosci Biobehav Rev 15:185–215
11. **Grunfeld C, Feingold K** 1992 Metabolic disturbances and wasting in the acquired immuno-deficiency syndrome. N Engl J Med 327: 329–337
12. **Kalra SP, Sahu A, Dube MG, Bonavera JJ, Kalra PS** 1996 Neuropeptide Y and its neural connections in the etiology of obesity and associated neuroendocrine and behavioral disorders. In: Bray GA, Ryan DH (eds) Molecular and Genetic Aspects of Obesity. LSU Press, Baton Rouge, LA, vol. 5:219–232
13. **Morely JE** 1987 Neuropeptide regulation of appetite and weight. Endocr Rev 8:256–287
14. **Motta M** 1991 Brain Endocrinology. Raven Press, New York, pp. 1–483
15. **Kalra SP, Kalra PS** 1996 Nutritional infertility: the role of the interconnected hypothalamic ($\gamma$-galanin-opioid network. Front Neuroendocrinol 17:371–401
16. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Freidman J** 1994 Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432
17. **Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chaib BT, Rabanowitz D, Lallone RL, Burley SK, Friedman JM** 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543–546
18. **Kalra PS, Dube MG, Xu B, Kalra SP,** Increased neuropeptide Y (NPY)-receptor sensitivity may underlie hyperphagia and obesity: a novel mechanism. Program of the Annual Conference of the North American Association for the Study of Obesity, Breckenridge, CO, 1996
19. **Kalra PS, Dube MG, Xu B, Farmerie WG, Kalra SP** 1998 Neuropeptide Y (NPY) $Y_1$ receptor mRNA is upregulated in association with transient hyperphagia and body weight gain: evidence for a

hypothalamic site for concurrent development of leptin resistance. J Neuroendocrinol 10:43–49
20. **Hökfelt T, Füxe K, Pernow B** 1986 Coexistence of neuronal messengers: a new principle in chemical neurotransmission. Progr Brain Res 68:1–411
21. **Everitt BJ, Hökfelt T** 1989 The coexistence of neuropeptide Y with other peptides and amines in the central nervous system. In: Mutt V, Füxe K, Hökfelt T, Lundberg J (eds) Neuropeptide Y. Raven Press, New York pp 61–72
22. **Holets VR, Hökfelt T, Rökaeus Å, Terenius L, Goldstein M** 1988 Locus coeruleus neurons in the rat containing neuropeptide Y, tyrosine hydroxylase, or galanin and their efferent projections to the spinal cord, cerebral cortex and hypothalamus. Neuroscience 24:893–906
23. **Kalra SP, Horvath T, Naftolin F, Xu B, Pu S, Kalra PS** 1997 The interactive language of the hypothalamus for the gonadotropin releasing hormone (GNRH) system. J Neuroendocrinol 9:569–576
24. **Brobeck JR** 1946 Mechanism of the development of obesity in animals with hypothalamic lesions. Physiol Rev 26:541–559
25. **Anand BK, Brobeck JR** 1951 Hypothalamic control of food intake in rats and cats. Yale J Biol Med 24:123–146
26. **Powley TL, Opsahl CH, Cox JE, Weingarten HP** 1980 The role of the hypothalamus in energy homeostasis. In: Morgane PJ, Panksepp J (eds) Handbook of the Hypothalamus. Part A: Behavioral Studies of the Hypothalamus. Marcel Dekker, Inc., New York, vol 3:211–298
27. **Luiten PG, ter Horst GJ, Steffens AB** 1987 The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. Prog Neurobiol 28:1–54
28. **Sclafani A, Kirchgessner A** 1986 The role of the medial hypothalamus in the control of food intake: an update. In: Ritter RC, Ritter S, Barnes CD (eds) Feeding Behavior. Academic Press, New York, pp 27–66
29. **Bray GA** 1984 Hypothalamic and genetic obesity: an appraisal of the autonomic hypothesis and the endocrine hypothesis. J Obes 8[Suppl 1]:119–137
30. **Sclafani A** 1971 Neural pathways involved in the ventromedial hypothalamic lesion syndrome in the rat. J Comp Physiol Psychol 77:70–96
31. **Kalra SP, Kalra PS** 1990 Neuropeptide Y: a novel peptidergic signal for the control of feeding behavior. In: Pfaff DW, Ganten D (eds) Current Topics in Neuroendocrinology. Springer-Verlag, Berlin, vol 10:192–197
32. **Leibowitz SF** 1989 Hypothalamic neuropeptide Y, galanin, and amines. Concepts of coexistence in relation to feeding behavior. Ann NY Acad Sci 575:221–233
33. **Horvath TL, Naftolin F, Leranth C, Sahu A, Kalra SP** 1996 Morphological and pharmacological evidence for neuropeptide Y-galanin interaction in the rat hypothalamus. Endocrinology 137: 3069–3077
34. **Horvath TL, Naftolin F, Kalra SP, Leranth C** 1992 Neuropeptide-Y innervation of $\beta$-endorphin-containing cells in the rat mediobasal hypothalamus: a light and electron microscopic double immunostaining analysis. Endocrinology 131:2461–2467
35. **Horvath TL, Kalra SP, Naftolin F, Leranth C** 1995 Morphological evidence for a galanin-opiate interaction in the rat mediobasal hypothalamus. J Neuroendocrinol 7:579–588
36. **Horvath TL, Bechmann I, Naftolin F, Kalra SP, Leranth C** 1997 Heterogeneity in the neuropeptide Y-containing neurons of the rat arcuate nucleus: GABAergic and non-GABAergic subpopulations. Brain Res 756:283–286
37. **Pu S, Jain M, Horvath TL, Diano S, Kalra SP,** Co-existence of neuropeptide Y (NPY) and $\gamma$-aminobutyric acid (GABA) in the hypothalamus: interactive effects on feeding. Program of the 27th Annual Meeting of the Society for Neuroscience, New Orleans, LA, 1997, vol 23:1344 (Abstract)
38. **Pu S, Jain MR, Horvath TL, Diano S, Kalra PS, Kalra SP** 1999 Interactions between neuropeptide Y and $\gamma$-aminobutyric acid in stimulation of feeding: a morphological and pharmacological analysis. Endocrinology 140:933–940
39. **Hahn TM, Breiniger JF, Baskin DG, Schwartz MW** 1998 Coexpression of AgrP and NPY in fasting-activated hypothalamic neurons. Nat Neurosci 1:271–272
40. **Chen P, Li C, Haskell-Luevano C, Cone RD, Smith MS,** 1998 Expression of agouti-related transcript (ART) and neuropeptide Y (NPY) in the hypothalamic arcuate nucleus of female rats during the estrous cycle. Annual Meeting of the Endocrine Society, New Orleans, LA, 1998 (Abstract P3–663), p 521
41. **Chronwall BM** 1985 Anatomy and physiology of the neuroendocrine arcuate nucleus. Peptides 6 [Suppl 2]:-11
42. **Finley JC, Maderdrut JL, Petrusz P** 1981 The immunocytochemical localization of enkephalin in the central nervous system of the rat. J Comp Neurol 198:541–565
43. **Finley JC, Lindstrom P, Petrusz P** 1981 Immunocytochemical localization of beta-endorphin-containing neurons in the rat brain. Neuroendocrinology 33:28–42
44. **Khachaturian H, Lewis ME, Shafer MKH, Watson SJ** 1985 Anatomy of CNS opioid system. Trends Neurosci 8:111–119
45. **Merchenthaler I, Lopez FJ, Negro-Vilar A** 1993 Anatomy and physiology of central galanin-containing pathways. Prog Neurobiol 40:711–769
46. **Crawley JN** 1995 Biological actions of galanin. Regul Pept 59:1–16
47. **Decavel C, van den Pol AN** 1990 GABA: a dominant neurotransmitter in the hypothalamus. J Comp Neurol 302:1019–1037
48. **Meister B, Hökfelt T** 1988 Peptide- and transmitter-containing neurons in the mediobasal hypothalamus and their relation to GABAergic systems: possible roles in control of prolactin and growth hormone secretion. Synapse 2:585–605
49. **van den Pol AN, Wuarin JP, Dudek FE** 1990 Glutamate, the dominant excitatory transmitter in neuroendocrine regulation. Science 250:1276–1278
50. **van den Pol AN** 1991 Glutamate and aspartate immunoreactivity in hypothalamic presynaptic axons. J Neurosci 11:2087–2101
51. **Smith AI, Funder JW** 1988 Proopiomelanocortin processing in the pituitary, central nervous system, and peripheral tissues. Endocr Rev 9:159–179
52. **Eipper BA, Mains RE** 1980 Structure and biosynthesis of proadrenocorticotropin/endorphin and related peptides. Endocr Rev 1:1–27
53. **Imura H, Kato Y, Nakai Y, Nakao K, Tanaka I, Jingami H, Koh T, Yoshimasa T, Tsukada T, Suda M, Nakao K, Jinganii H, Koh T, Yihimago T, Tseukada T, Suda M, Sakanooto M, Morii N, Takesheshi H, Tojo K, Sugawara A** 1985 Endogenous opioids and related peptides: from molecular biology to clinical medicine. The Sir Henry Dale lecture for 1985. J Endocrinol 107:147–157
54. **Shutter JR, Graham M, Kinsey AC, Scully S, Luthy R, Stark KL** 1997 Hypothalamic expression of ART, a novel gene related to agouti, is upregulated in obese and diabetic mutant mice. Genes Dev 11:593–602
55. **Ollmann MM, Wilson BD, Yang YK, Kerns JA, Chen Y, Gantz I, Barsh GS** 1997 Antagonism of central melanocortin receptors in *in vitro* and *in vivo* by agouti-related protein. Science 278:135–138
56. **Kalra PS, Norlin M, Kalra SP** 1995 Neuropeptide Y stimulates beta-endorphin release in the basal hypothalamus: role of gonadal steroids. Brain Res 705:353–356
57. **Douglass J, McKinzie AA, Couceryo P** 1995 PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. J Neurosci 15:2471–2481
58. **Gautvik KM, de Lecea L, Gautvik VT, Danielson PE, Tranque P, Dopazo A, Bloom FE, Sutcliffe JG** 1996 Overview of the most prevalent hypothalamus-specific mRNA, as identified by directional tag PCR subtraction. Proc Natl Acad Sci USA 93:8733–8738
59. **Koylu EO, Couceyro PR, Lambert PD, Ling NC, De Souza EB, Kuhar MJ** 1997 Immunohistochemical localization of novel CART peptides in the rat hypothalamus, pituitary and adrenal gland. J Neuroendocrinol 9:823–833
60. **Lambert PD, Couceyro PR, McGirr KM, Dall Vechia SE, Smith Y, Kuhar MJ** 1998 CART peptides in the central control of feeding and interactions with neuropeptide Y. Synapse 29:293–298
61. **Kristensen P, Judge ME, Thim L, Ribel U, Christjansen KN, Wulff BS, Clausen JT, Jensen PB, Madsen OD, Vrang N, Larsen PJ, Hastrup S** 1998 Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 393:72–76
62. **Kalra PS, Dube MG, Xu B, Farmerie WG, Kalra SP** 1998 Evidence

that dark-phase hyperphagia induced by neurotoxin 6-hydroxydopamine may be due to decreased leptin and increased NPY signaling. Physiol Behav 63:829–835
63. **Kalra PS, Dube MG, Xu B, Kalra SP** 1997 Increased receptor sensitivity to neuropeptide Y in the hypothalamus may underlie transient hyperphagia and body weight gain. Regul Pept 72:121–130
64. **Kalra PS, Edwards TG, Dube MG** 1999 Hypothalamic galanin is upregulated during hyperphagia. NY Acad Sci 863:432–434
65. **Jain MR, Dube MG, Kalra SP, Kalra PS** 1998 Neuropeptide Y release in the paraventricular nucleus is decreased during transient hyperphagia induced by microinjection of colchicine into the ventromedial nucleus of rats. Neurosci Lett 256:21–24
66. **Pu S, Dube MG, Xu B, Kalra SP, Kalra PS,** Microinjection of colchicine into the hypothalamic ventromedial nucleus upregulates galanin expression in association with transient hyperphagia. Program of the 28th Annual Meeting of the Society for Neuroscience, Los Angeles, CA, 1998, vol 24: 448 (Abstract 175.8)
67. **Pu S, Dube MG, Edwards TG, Kalra SP, Kalra PS** 1999 Disruption of neural signaling within the hypothalamic ventromedial nucleus upregulates galanin gene expression in association with hyperphagia: an *in situ* analysis. Mol Brain Res, in press
68. **Pu S, Dube MG, Xu B, Kalra SP, Kalra PS,** Induction of neuropeptide Y (NPY) gene expression in novel hypothalamic sites in association with transient hyperphagia and body weight gain. Program of the 80th Annual Meeting of The Endocrine Society, New Orleans, LA, 1998 (Abstract P3–236), p 435
69. **Shimizu N, Oomura Y, Plata-Salaman CR, Morimoto M** 1987 Hyperphagia and obesity in rats with bilateral ibotenic acid-induced lesions of the ventromedial hypothalamic nuclei. Brain Res 416:153–156
70. **Stanley BG, Chin AS, Leibowitz SF** 1985 Feeding and drinking elicited by central injection of neuropeptide Y: evidence for a hypothalamic site(s) of action. Brain Res Bull 14:521–524
71. **Kyrkouli SE, Stanley BG, Seirafi RD, Leibowitz SF** 1990 Stimulation of feeding by galanin: anatomical localization and behavioral specificity of this peptide's effects in the brain. Peptides 11:995–1001
72. **Kyrkouli SE, Stanley BG, Leibowitz SF** 1986 Galanin: stimulation of feeding induced by medial hypothalamic injection of this novel peptide. Eur J Pharmacol 122:159–160
73. **Schick RR, Samsami S, Zimmermann JP, Eberl T, Endres C, Schusdziarra V, Classen M** 1993 Effect of galanin on food intake in rats: involvement of lateral and ventromedial hypothalamic sites. Am J Physiol 264:R355–R361
74. **Grandison L, Guidotti A** 1977 Stimulation of food intake by muscimol and beta endorphin. Neuropharmacology 16:533–536
75. **Kelly J, Grossman SP** 1980 GABA and hypothalamic feeding systems. Brain Res Bull 5:237–244
76. **Kelly J, Rothstein J, Grossman SP** 1979 GABA and hypothalamic feeding systems. I. Topographic analysis of the effects of microinjections of muscimol. Physiol Behav 23:1123–1134
77. **Kelly J, Alheid GF, Newberg A, Grossman SP** 1977 GABA stimulation and blockade in the hypothalamus and midbrain: effects on feeding and locomotor activity. Pharmacol Biochem Behav 7:537–541
78. **Satoh N, Ogawa Y, Katsuura G, Hayase M, Tsuji T, Imagawa K, Yoshimasa Y, Nishi S, Hosoda K, Nakao K** 1997 The arcuate nucleus as a primary site of satiety effect of leptin in rats. Neurosci Lett 224:149–152
79. **Jacob RJ, Dziura J, Medwick MB, Leone P, Caprio S, During M, Shulman GI, Sherwin RS** 1997 The effect of leptin is enhanced by microinjection into the ventromedial hypothalamus. Diabetes 46: 150–152
80. **Ohata H, Suzuki K, Oki Y, Arai K, Shibasaki T,** Urocortin in the ventromedial nucleus of the hypothalamus (VMH) inhibits feeding behavior in rats. Program of the 80th Annual Meeting of The Endocrine Society, New Orleans, LA, 1998 (Abstract OR22–2), p 95
81. **Bernardis LL, Bellinger LL** 1987 The dorsomedial hypothalamic nucleus revisited: 1986 update. Brain Res 434:321–381
82. **Bellinger LL, Mendel VE, Bernardis LL, Castonguay TW** 1986 Meal patterns of rats with dorsomedial hyopthalamic nuclei lesions or sham operations. Physiol Behav 36:693–698
83. **Moga M, Saper CB** 1994 Neuropeptide-immunoreactive neurons projecting to the paraventricular hypothalamic nucleus in the rat. J Comp Neurol 346:137–150
84. **Thompson RH, Canteras NS, Swanson LW** 1996 Organization of projections from the dorsomedial nucleus of the hypothalamus: a PHA-L study in the rat. J Comp Neurol 376:143–173
85. **Bernardis LL, Bellinger LL** 1996 The lateral hypothalamic area revisited: ingestive behavior. Neurosci Biobehav Rev 20:189–287
86. **Steffens AB** 1975 Influence of reversible obesity on eating behavior, blood glucose, and insulin in the rat. Am J Physiol 281:1738–1744
87. **Steinbaum EA, Miller NE** 1965 Obesity from eating elicited by daily stimulation of hypothalamus. Am J Physiol 208:1–5
88. **Elmquist JK, Ahima RS, Maratos-Flier E, Flier JS, Saper CB** 1997 Leptin activates neurons in ventrobasal hypothalamus and brainstem. Endocrinology 138:839–842
89. **Yokosuka M, Xu B, Pu S, Kalra SP,** The magnocellular part of the paraventricular hypothalamic nucleus (PVN): a selective site for inhibition of NPY-induced food intake by leptin. Program of the 27th Annual Meeting Society for Neuroscience, New Orleans, LA, 1997, vol 23:1344 (Abstract)
90. **Yokosuka M, Xu B, Pu S, Kalra PS, Kalra SP** 1998 Neural substrates for leptin and neuropeptide Y (NPY) interaction: hypothalamic sites associated with inhibition of NPY-induced food intake. Physiol Behav 64:331–338
91. **Sawchenko PE, Swanson LW** 1983 The organization of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. J Comp Neurol 218:121–144
92. **Elmquist JK, Ahima RS, Elias CF, Flier JS, Saper CB** 1998 Leptin activates distinct projections from the dorsomedial and ventromedial hypothalamic nuclei. Proc Natl Acad Sci USA 95:741–746
93. **Bai FL, Yamano M, Shiotani Y, Emson PC, Smith AD, Powell JF, Tohyama M** 1985 An arcuato-paraventricular and -dorsomedial hypothalamic neuropeptide Y-containing system which lacks noradrenaline in the rat. Brain Res 331:172–175
94. **Sahu A, Kalra PS, Kalra SP** 1988 Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. Peptides 9:83–86
95. **Kamegai J, Minami S, Sugihara H, Hasegawa O, Higuchi H, Wakabayashi I** 1996 Growth hormone receptor gene is expressed in neuropeptide Y neurons in hypothalamic arcuate nucleus of rats. Endocrinology 137:2109–2112
96. **Chan YY, Steiner RA, Clifton DK** 1996 Regulation of hypothalamic neuropeptide-Y neurons by growth hormone in the rat. Endocrinology 137:1319–1325
97. **Kesterson RA, Huszar D, Lynch CA, Simerly RB, Cone RD** 1997 Induction of neuropeptide Y gene expression in the dorsal medial hypothalamic nucleus in two models of the agouti obesity syndrome. Mol Endocrinol 11:630–637
98. **Li C, Chen P, Smith MS** 1998 Neuropeptide Y (NPY) neurons in the arcuate nucleus (ARH) and dorsomedial nucleus (DMH), areas activated during lactation, project to the paraventricular nucleus of the hypothalamus. Regul Pept 75–76:93–100
99. **Swanson LW, Sawchenko PE** 1983 Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. Annu Rev Neurosci 6:269–324
100. **Dube MG, Kalra SP, Kalra PS,** Food intake elicited by central administration of orexins: identification of hypothalamic sites of action. Program of the 28th Annual Meeting of the Society for Neuroscience, Los Angeles, CA, 1998, vol 24:448 (Abstract 175.8)
101. **Krahn DD, Gosnell BA, Levine AS, Morley JE** 1988 Behavioral effects of corticotropin-releasing factor: localization and characterization of central effects. Brain Res 443:63–69
102. **Monnikes H, Heymann-Monnikes I, Tache Y** 1992 CRF in the paraventricular nucleus of the hypothalamus induces dose-related behavioral profile in rats. Brain Res 574:70–76
103. **Li BH, Xu B, Rowland NE, Kalra SP** 1994 c-fos Expression in the rat brain following central administration of neuropeptide Y and effects of food consumption. Brain Res 665:277–284
104. **Xu B, Li BH, Rowland NE, Kalra SP** 1995 Neuropeptide Y injection into the fourth cerebroventricle stimulates c-fos expression in the paraventricular nucleus and other nuclei in the forebrain: effect of food consumption. Brain Res 698:227–231

105. **Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG** 1996 Identification of targets of leptin action in rat hypothalamus. J Clin Invest 98:1101–1106
106. **Kalra SP, Dube MG, Sahu A, Phelps CP, Kalra PS** 1991 Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. Proc Natl Acad Sci USA 88:10931–10935
107. **Dube MG, Sahu A, Kalra PS, Kalra SP** 1992 Neuropeptide Y release is elevated from the microdissected paraventricular nucleus of food-deprived rats: an *in vitro* study. Endocrinology 131:684–688
108. **Aravich PF, Sclafani A** 1983 Paraventricular hypothalamic lesions and medial hypothalamic knife cuts produce similar hyperphagia syndromes. Behav Neurosci 97:970–983
109. **Weingarten HP, Chang PK, McDonald TJ** 1985 Comparison of the metabolic and behavioral disturbances following paraventricular- and ventromedial-hypothalamic lesions. Brain Res Bull 14:551–559
110. **Tokunaga K, Fukushima M, Kemnitz JW, Bray GA** 1986 Comparison of ventromedial and paraventricular lesions in rats that become obese. Am J Physiol 251:R1221–R1227
111. **Leibowitz SF** 1978 Paraventricular nucleus: a primary site mediating adrenergic stimulation of feeding and drinking. Pharmacol Biochem Behav 8:163–175
112. **Stanley BG, Magdalin W, Seirafi A, Thomas WJ, Leibowitz SF** 1993 The perifornical area: the major focus of (a) patchily distributed hypothalamic neuropeptide Y-sensitive feeding system(s). Brain Res 604:304–317
113. **Stricker EM** 1990 Handbook of Behavioral Neurobiology: Neurobiology of Food and Fluid Intake. Plenum Press, New York, vol 10:1–553
114. **Nagai K, Nishio T, Nakagawa H, Nakamura S, Fukuda Y** 1978 Effect of bilateral lesions of the suprachiasmatic nuclei on the circadian rhythm of food-intake. Brain Res 142:384–389
115. **Van den Pol AN, Powley T** 1979 A fine-grained anatomical analysis of the role of the rat suprachiasmatic nucleus in circadian rhythms of feeding and drinking. Brain Res 160:307–326
116. **Leon GR, Roth L** 1977 Obesity: psychological causes, correlations, and speculations. Psychol Bull 84:117–139
117. **Jhanwar-Uniyal M, Beck B, Burlet C, Leibowitz SF** 1990 Diurnal rhythm of neuropeptide Y-like immunoreactivity in the suprachiasmatic, arcuate and paraventricular nuclei and other hypothalamic sites. Brain Res 536:331–334
118. **Akabayashi A, Zaia CT, Koenig JI, Gabriel SM, Silva I, Leibowitz SF** 1994 Diurnal rhythm of galanin-like immunoreactivity in the paraventricular and suprachiasmatic nuclei and other hypothalamic areas. Peptides 15:1437–1444
119. **Akabayashi A, Watanabe Y, Gabriel SM, Chae HJ, Leibowitz SF** 1994 Hypothalamic galanin-like immunoreactivity and its gene expression in relation to circulating corticosterone. Brain Res Mol Brain Res 25:305–312
120. **Xu B, Kalra PS, Kalra SP,** Food restriction upregulates hypothalamic NPY gene expression: loss of daily rhythmicity. Program of the 26th Annual Meeting of the Society for Neuroscience, Washington DC, 1996, vol 22:1685 (Abstract)
121. **Xu B, Kalra PS, Kalra SP,** Food restriction abolishes the daily rhythm in gene expression of hypothalamic neuropeptide Y (NPY), galanin (GAL) and $\beta$-endorphin (POMC) and adipocyte leptin but not in leptin secretions. Program of the 80th Annual Meeting of The Endocrine Society, New Orleans, 1998 (Abstract P3–246), p 437
122. **Steiner RA, Kabigting E, Lent K, Clifton DK** 1994 Diurnal rhythm in proopiomelanocortin mRNA in the arcuate nucleus of the male rat. J Neuroendocrinol 6:603–608
123. **Xu B, Kalra PS, Farmerie WG, Kalra SP,** Daily changes in hypothalamic gene expression of neuropeptide Y, galanin, proopiomelanocortin and adipocyte leptin gene expression and secretion: effects of food-restriction. Endocrinology, in press
124. **Buijs RM** 1996 The anatomical basis for the expression of circadian rhythms: the efferent projections of the suprachiasmatic nucleus. Prog Brain Res 111:229–240
125. **Watts AG, Swanson LW, Sanchez-Watts G** 1987 Efferent projections of the suprachiasmatic nucleus. I. Studies using anterograde transport of Phaseolus vulgaris leucoagglutinin in the rat. J Comp Neurol 258:204–229
126. **Watts AG, Swanson LW** 1987 Efferent projections of the suprachiasmatic nucleus. II. Studies using retrograde transport of fluorescent dyes and simultaneous peptide immunohistochemistry in the rat. J Comp Neurol 258:230–252
127. **Vrang N, Larsen PJ, Mikkelsen JD** 1995 Direct projection from the suprachiasmatic nucleus to hypophysiotrophic corticotropin-releasing factor immunoreactive cells in the paraventricular nucleus of the hypothalamus demonstrated by means of Phaseolus vulgaris-leucoagglutinin tract tracing. Brain Res 684:61–69
128. **Hermes ML, Buijs RM, Renaud LP** 1996 Electrophysiology of suprachiasmatic nucleus projections to hypothalamic paraventricular nucleus neurons. Prog Brain Res 111:241–252
129. **Luiten PG, ter Horst GJ, Steffens AB** 1987 The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. Prog Neurobiol 28:1–54
130. **Horvath TL** 1997 Suprachiasmatic efferents avoid phenestrated capillaries but innervate neuroendocrine cells, including those producing dopamine. Endocrinology 138:1312–1320
131. **Akabayashi A, Levin N, Paez X, Alexander JT, Leibowitz SF** 1994 Hypothalamic neuropeptide Y and its gene expression: relation to light/dark cycle and circulating corticosterone. Mol Cell Neurosci 5:210–218
132. **Buijs RM, Markman M, Nunes-Cardoso B, Hou YX, Shinn S** 1993 Projections of the suprachiasmatic nucleus to stress-related areas in the rat hypothalamus: a light and electron microscopic study. J Comp Neurol 335:42–54
133. **Roland BL, Sawchenko PE** 1993 Local origins of some GABAergic projections to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. J Comp Neurol 332:123–143
134. **Levin MC, Sawchenko PE, Howe PR, Bloom SR, Polak JM** 1987 Organization of galanin-immunoreactive inputs to the paraventricular nucleus with special reference to their relationship to catecholaminergic afferents. J Comp Neurol 261:562–582
135. **ter Horst GJ, Luiten PG** 1986 The projections of the dorsomedial hypothalamic nucleus in the rat. Brain Res Bull 16:231–248
136. **Clark JT, Kalra PS, Crowley WR, Kalra SP** 1984 Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. Endocrinology 115:427–429
137. **Clark JT, Sahu A, Kalra PS, Balasubramaniam A, Kalra SP** 1987 Neuropeptide Y (NPY)-induced feeding behavior in female rats: comparisons with human NPY ([Met17]NPY), NPY analog ([norLeu4]NPY) and peptide YY. Regul Pept 17:31–39
138. **Tatemoto K** 1982 Neuropeptide Y: complete amino acid sequence of the brain peptide. Proc Natl Acad Sci USA 79:5485–5489
139. **Kalra SP, Kalra PS** 1996 Is neuropeptide Y a naturally occurring appetite transducer? Curr Opin Endocrinol Diab 3:157–163
140. **Chronwall BB** 1988 Anatomical distribution of NPY and NPY messenger RNA in the brain. In: Mutt V, Füxe K, Hökfelt T, Lundberg JD (eds) Neuropeptide Y. Raven Press, New York, pp. 51–60
141. **Sahu A, Dube MG, Kalra SP, Kalra PS** 1988 Bilateral neural transection at the level of mesencephalon increase food intake and reduce latency to onset of feeding in response to neuropeptide Y. Peptides 9:1269–1273
142. **Sahu A, Kalra SP, Crowley WR, Kalra PS** 1988 Evidence that NPY-containing neurons in the brain stem project into selected hypothalamic nuclei: implication in feeding behavior. Brain Res 457:376–378
143. **Clark JT, Gist RS, Kalra SP, Kalra PS** 1988 Alpha 2-adrenoceptor blockade attenuates feeding behavior induced by neuropeptide Y and epinephrine. Physiol Behav 43:417–422
144. **Baker RA, Herkenham M** 1995 Arcuate nucleus neurons that project to the hypothalamic paraventricular nucleus: neuropeptidergic identity and consequences of adrenalectomy on mRNA levels in the rat. J Comp Neurol 358:518–530
145. **Dube MG, Sahu A, Phelps CP, Kalra PS, Kalra SP** 1992 Neuropeptide Y (NPY) concentration is decreased in the paraventricular nucleus (PVN) of rats exhibiting excessive weight gain produced by ventromedial hypothalamic lesions. Program of the 22nd Annual Meeting of the Society for Neuroscience, Anaheim, CA, 1992, vol 18:937 (Abstract)
146. **Dube MG, Xu B, Kalra PS, Sninsky CA, Kalra SP** 1999 Disruption

in neuropeptide Y and leptin signaling in obese ventromedial hypothalamic-lesioned rats. Brain Res 816:38–46
147. **Dumont T, Jacques D, St. Pierre J-A, Quirion R** 1997 Neuropeptide Y receptor types in the mammalian brain: species differences and status in the human central nervous system. In: Grundermar L, Bloom S (eds) Neuropeptide Y and Drug Development. Academic Press, New York, pp 57–86
148. **Mikkelsen JD, Larsen PJ** 1992 A high concentration of NPY ($Y_1$)-receptor mRNA-expressing cells in the rat arcuate nucleus. Neurosci Lett 148:195–198
149. **Larsen PJ, Sheikh SP, Jakobsen CR, Schwartz TW, Mikkelsen JD** 1993 Regional distribution of putative NPY $Y_1$ receptors and neurons expressing $Y_1$ mRNA in forebrain areas of the rat central nervous system. Eur J Neurosci 5:1622–1637
150. **Daniels AJ, Matthews JE, Slepetis RJ, Jansen M, Viveros OH, Tadepalli A, Harrington W, Heyer D, Landavazo A, Leban JJ, Spaltenstein A** 1995 High-affinity neruopeptide Y receptor antagonists. Proc Natl Acad Sci USA 92:9067–9071
151. **Matthews J, Jansen M, Lyarly D, Cox R, Chen W-J, Koller K, Daniels AJ** 1997 Pharmacological characterization and selectivity of the NPY antagonist GR231118 (1229U91) for different NPY receptors. Regul Pept 72:113–119
152. **Kanatani A, Ishihara A, Asahi S, Tanaka T, Ozaki S, Ihara M** 1996 Potent neuropeptide Y $Y_1$ receptor antagonist, 1229U91: blockade of neuropeptide Y-induced and physiological food intake. Endocrinology 137:3177–3182
153. **Lopez-Valpuesta FJ, Nyce JW, Griffin-Biggs TA, Ice JC, Meyers RD** 1996 Antisense to NPY $Y_1$ demonstrates that $Y_1$ receptors in the hypothalamus underlie NPY hypothermia and feeding in rats. Proc R Soc Lond B Biol Sci 263:881–886
154. **Li DL, Simmons RMA, Bruns RF, Gehlert DR, Hipskind PA, Kaliman D, Helton R, Aaron E, Schober D, Iyenger S,** Effects of the neuropeptide Y $Y_1$ antagonist BIBP3226 and 1229U91 on NPY-induced feeding in CD-1 mice. Program of the 27th Annual Meeting of the Society for Neuroscience, New Orleans, LA, 1997, vol 23:1766 (Abstract)
155. **Iyenger S, Simmons RMA, Li DL, Cantrell B, Kallman MJ, Bruns RF, Calligaro DO, Hipskind PA, Zimmerman DM, Gehlert DR,** Effect of new structurally novel neuropeptide Y antagonists on NPY-induced feeding. Program of the 27th Annual Meeting of the Society for Neuroscience, New Orleans, LA, 1997, vol 23:1766 (Abstract)
156. **Gerald C, Walker MW, Criscione L, Gustafson EL, Batzl-Hartmann C, Smith KE, Vaysse P, Durkin MM, Laz TM, Linemeyer DL, Schaffhauser AO, Whitebread S, Hofbauer KG, Taber RI, Branchek TA, Weinshank RL** 1996 A receptor subtype involved in neuropeptide Y-induced food intake. Nature 382:168–171
157. **Hu Y, Bloomquist BT, Cornfield LJ, DeCarr LB, Flores-Riveros JR, Friedman L, Jiang P, Lewis-Higgins L, Sadlowski Y, Schaefer J, Velazquez N, McCaleb ML** 1996 Identification of a novel hypothalamic neuropeptide Y receptor associated with feeding behavior. J Biol Chem 271:26315–26319
158. **Schaffhauser AO, Stricker-Krongrad A, Brunner L, Cumin F, Gerald C, Whitebread S, Criscione L, Hofbauer KG** 1997 Inhibition of food intake by neuropeptide Y $Y_5$ receptor antisense oligodeoxynucleotides. Diabetes 46:1792–1798
159. **Criscione L, Rigollier P, Bazl-Harmann C, Rüeger H, Stricker-Krongrad A, Whitebread S, Yamaguchi Y, Walker MW, Gerald C, Chiesi M, Levens N, Schilling W, Hofbauer KG** 1997 Inhibition of food intake in rodents induced by a selective antagonist of the neuropeptide Y $Y_5$ receptor subtype. Program of the 27th Annual Meeting of the Society for Neuroscience, New Orleans, LA, 1997, vol 23:575 (Abstract)
160. **Widdowson PS** 1997 Regionally-selective down-regulation of NPY receptor subtypes in the obese Zucker rat. Relationship to the Y5 "feeding" receptor. Brain Res 758:17–25
161. **Dumont Y, Fournier A, Quirion R** 1998 Expression and characterization of the neuropeptide Y Y5 receptor subtype in the rat brain. J Neurosci 18:5565–5574
162. **Füxe K, Agnati LH (eds)** 1991 Volume Transmission in the Brain: New Mechanism for Neural Transmission, vol 1: Advances in neuroscience. Raven Press, New York
163. **Füxe K, Agnati L, Aguirre J, Bjelke B, Tinner E, Merlo P, Eneroth P** 1991 On the existence of volume transmission in the central neuropeptide Y receptor mismatch and on biological effects of neuropeptide Y fragments. In: Füxe K, Agnati LF (eds) Volume Transmission in the Brain: Novel Mechanisms for Neural Transmission. Raven Press, New York, vol. 1:105–130
164. **Yokusuka M, Xu B, Kalra PS, Kalra SP,** Neuropeptide Y target neurons for stimulation of feeding reside in the magnocellular paraventricular nucleus. Program of the 28th Annual Meeting of the Society for Neuroscience. Los Angeles, CA, 1998, vol 24:11 (Abstract 11.2)
165. **Meister B, Ceccatelli S, Hökfelt T, Anden NE, Anden M, Theodorsson E** 1989 Neurotransmitters, neuropeptides and binding sites in the rat mediobasal hypothalamus: effects of monosodium glutamate (MSG) lesions. Exp Brain Res 76:343–368
166. **Broberger C, Landry M, Wong H, Walsh J, Hökfelt T** 1997 Subtypes $Y_1$ and $Y_2$ of the neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin- and neuropeptide-Y-containing neurons of the rat hypothalamic arcuate nucleus. Neuroendocrinology 66:393–408
167. **Füxe K, Tinner B, Caberlotto L, Bunnemann B, Agnati LF** 1997 NPY $Y_1$ receptor-like immunoreactivity exists in a subpopulation of beta-endorphin immunoreactive nerve cells in the arcuate nucleus: a double immunolabeling analysis in the rat. Neurosci Lett 225:49–52
168. **Rhim H, Kinney GA, Emmerson PJ, Miller RJ** 1997 Regulation of neurotransmission in the arcuate nucleus of the rat by different neuropeptide Y receptors. J Neurosci 17:2980–2989
169. **Clark JT, Kalra PS, Kalra SP** 1985 Neuropeptide Y stimulates feeding but inhibits sexual behavior in rats. Endocrinology 117: 2435–2442
170. **Kalra SP, Dube MG, Kalra PS** 1988 Continuous intraventricular infusion of neuropeptide Y evokes episodic food intake in satiated female rats: effects of adrenalectomy and cholecystokinin. Peptides 9:723–728
171. **Kalra SP, Clark JT, Sahu A, Dube MG, Kalra PS** 1988 Control of feeding and sexual behaviors by neuropeptide Y: physiological implications. Synapse 2:254–257
172. **Dube MG, Xu B, Crowley WR, Kalra PS, Kalra SP** 1994 Evidence that neuropeptide Y is a physiological signal for normal food intake. Brain Res 646:341–344
173. **Xu B, Kalra PS, Moldawer LL, Kalra SP** 1998 Increased appetite augments hypothalamic NPY Y1 receptor gene expression: effects of anorexigenic ciliary neurotropic factor. Regul Pept 75–76:391–395
174. **Dryden S, Pickavance L, Frankish HM, Williams G** 1995 Increased neuropeptide Y secretion in the hypothalamic paraventricular nucleus of obese (fa/fa) Zucker rats. Brain Res 690:185–188
175. **Sancora G, Kershaw M, Finkelstein JA, White JD** 1990 Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. Endocrinology 127:730–737
176. **Wilding JP, Gilbey SG, Bailey CJ, Batt RA, Williams G, Ghatei MA, Bloom SR** 1993 Increased neuropeptide-Y messenger ribonucleic acid (mRNA) and decreased neurotensin mRNA in the hypothalamus of the obese (ob/ob) mouse. Endocrinology 132: 1939–1944
177. **Williams G, Steel JH, Cardoso H, Ghatei MA, Lee YC, Gill JS, Burrin JM, Polak JM, Bloom SR** 1988 Increased hypothalamic neuropeptide Y concentrations in diabetic rat. Diabetes 37:763–772
178. **Williams G, Gill JS, Lee YC, Cardoso HM, Okpere BE, Bloom SR** 1989 Increased neuropeptide Y concentrations in specific hypothalamic regions of streptozocin-induced diabetic rats. Diabetes 38: 321–327
179. **Sahu A, Sninsky CA, Kalra PS, Kalra SP** 1990 Neuropeptide-Y concentration in microdissected hypothalamic regions and *in vitro* release from the medial basal hypothalamus-preoptic area of streptozotocin-diabetic rats with and without insulin substitution therapy. Endocrinology 126:192–198
180. **Sahu A, Sninsky CA, Phelps CP, Dube MG, Kalra PS, Kalra SP** 1992 Neuropeptide Y release from the paraventricular nucleus increases in association with hyperphagia in streptozotocin-induced diabetic rats. Endocrinology 131:2979–2985
181. **Sahu A, Sninsky CA, Kalra SP** 1997 Evidence that hypothalamic

neuropeptide Y gene expression and NPY levels in the paraventricular nucleus increase before the onset of hyperphagia in experimental diabetes. Brain Res 755:339–342
182. **Smith MS** 1993 Lactation alters neuropeptide-Y and proopiomelanocortin gene expression in the arcuate nucleus of the rat. Endocrinology 133:1258–1265
183. **Pedrazzani T, Seydoux J, Kunstner P, Aubert JF, Grouzmann E, Beermann F, Brunner HR** 1998 Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY $Y_1$ receptor. Nat Med 4:722–726
184. **Kalra SP, Dube MG, Sahu A, Phelps CP, Kalra PS** 1991 Structure-function analysis of stimulation of food intake by neuropeptide Y: effects of receptor agonists. Physiol Behav 50:5–9
185. **Marsh DJ, Hollopeter G, Kafer KE, Palmiter RD** 1998 Role of the $Y_5$ neuropeptide Y receptor in feeding and obesity. Nat Med 4:718–721
186. **Haynes AC, Arch JR, Wilson S, McClue S, Buckingham RE** 1998 Characterisation of the neuropeptide Y receptor that mediates feeding in the rat: a role for the Y5 receptor? Regul Pept 75–76:355–361
187. **Wyss P, Stricker-Krongrad A, Brunner L, Miller J, Crossthwaite A, Whitebread S, Criscione L** 1998 The pharmacology of neuropeptide Y (NPY) receptor-mediated feeding in rats characterizes better Y5 than Y1, but not Y2 or Y4 subtypes. Regul Pept 75–76: 363–371
188. **Kanatani A, Ito J, Ishihara A, Iwaasa H, Fukuroda T, Fukami T, MacNeil DJ, Van der Ploeg LH, Ihara M** 1998 NPY-induced feeding involves the action of a Y1-like receptor in rodents. Regul Pept 75–76:409–415
189. **Sahu A, White JD, Kalra PS, Kalra SP** 1992 Hypothalamic neuropeptide Y gene expression in rats on scheduled feeding regimen. Mol Brain Res 15:15–18
190. **Guy J, Pelletier G, Bosler O** 1988 Serotonin innervation of neuropeptide Y-containing neurons in the rat arcuate nucleus. Neurosci Lett 85:9–13
191. **Dube MG, Sahu A, Phelps CP, Kalra PS, Kalra SP** 1992 Effect of d-fenfluramine on neuropeptide Y concentration and release in the paraventricular nucleus of food-deprived rats. Brain Res Bull 29: 865–869
192. **Rogers P, McKibbin PE, Williams G** 1991 Acute fenfluramine administration reduces neuropeptide Y concentrations in specific hypothalamic regions of the rat: possible implications for the anorectic effect of fenfluramine. Peptides 12:251–255
193. **Dryden S, Frankish HM, Wang Q, Pickavance L, Williams G** 1996 The serotonergic agent fluoxetine reduces neuropeptide Y levels and neuropeptide Y secretion in the hypothalamus of lean and obese rats. Neuroscience 72:557–566
194. **Sar M, Sahu A, Crowley WR, Kalra SP** 1990 Localization of neuropeptide-Y immunoreactivity in estradiol-concentrating cells in the hypothalamus. Endocrinology 127:2752–2756
195. **Sahu A, Phelps CP, White JD, Crowley WR, Kalra SP, Kalra PS** 1992 Steroidal regulation of hypothalamic neuropeptide Y release and gene expression. Endocrinology 130:3331–3336
196. **Sahu A, Crowley WR, Kalra SP** 1994 Hypothalamic neuropeptide-Y gene expression increases before the onset of the ovarian steroid-induced luteinizing hormone surge. Endocrinology 134: 1018–1022
197. **Hisano S, Kagotani Y, Tsuruo Y, Daikoku S, Chihara K, Whitnall MH** 1988 Localization of glucocorticoid receptor in neuropeptide Y-containing neurons in the arcuate nucleus of the rat hypothalamus. Neurosci Lett 95:13–18
198. **Harfstrand A, Cintra A, Fuxe K, Aronsson M, Wikstrom AC, Okret S, Gustafsson JA, Agnati LF** 1989 Regional differences in glucocorticoid receptor immunoreactivity among neuropeptide Y immunoreactive neurons of the rat brain. Acta Physiol Scand 135: 3–9
199. **Larsen PJ, Jessop DS, Chowdrey HS, Lightman SL, Mikkelsen JD** 1994 Chronic administration of glucocorticoids directly upregulates prepro-neuropeptide Y and $Y_1$-receptor mRNA levels in the arcuate nucleus of the rat. J Neuroendocrinol 6:153–159
200. **Nicolaidis S** 1978 Neural mechanism of energy equilibrium. J Annu Diabetol Hotel Dieu 1:15:153–156
201. **Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte Jr D** 1992 Insulin in the brain: a hormonal regulator of energy balance. Endocr Rev 13:387–414
202. **Sahu A, Kalra SP, Crowley WR, Kalra PS** 1989 Testosterone raises neuropeptide Y concentrations in selected hypothalamic sites and *in vitro* release from the medial basal hypothalamus of castrated male rats. Endocrinology 124:410–414
203. **Sahu A, Crowley WR, Kalra SP** 1995 Evidence that hypothalamic neuropeptide Y gene expression increases before the onset of the preovulatory LH surge. J Neuroendocrinol 7:291–296
204. **Bonavera JJ, Dube MG, Kalra PS, Kalra SP** 1994 Anorectic effects of estrogen may be mediated by decreased neuropeptide-Y release in the hypothalamic paraventricular nucleus. Endocrinology 134: 2367–2370
205. **Baskin DG, Norwood BJ, Schwartz MW, Koerker DJ** 1995 Estradiol inhibits the increase of hypothalamic neuropeptide Y messenger ribonucleic acid expression induced by weight loss in ovariectomized rats. Endocrinology 136:5547–5554
206. **Liposits Z, Sievers L, Paull WK** 1988 Neuropeptide-Y and ACTH-immunoreactive innervation of corticotropin releasing factor (CRF)-synthesizing neurons in the hypothalamus of the rat. An immunocytochemical analysis at the light and electron microscopic levels. Histochemistry 88:227–234
207. **Wahlestedt C, Skagerberg G, Ekman R, Heilig M, Sundler F, Hakanson R** 1987 Neuropeptide Y (NPY) in the area of the hypothalamic paraventricular nucleus activates the pituitary-adrenocortical axis in the rat. Brain Res 417:33–38
208. **Wilding JP, Gilbey SG, Lambert PD, Ghatei MA, Bloom SR** 1993 Increases in neuropeptide Y content and gene expression in the hypothalamus of rats treated with dexamethasone are prevented by insulin. Neuroendocrinology 57:581–587
209. **White BD, Dean RG, Martin RJ** 1990 Adrenalectomy decreases neuropeptide Y mRNA levels in the arcuate nucleus. Brain Res Bull 25:711–715
210. **Akabayashi A, Watanabe Y, Wahlestedt C, McEwen BS, Paez X, Leibowitz SF** 1994 Hypothalamic neuropeptide Y, its gene expression and receptor activity: relation to circulating corticosterone in adrenalectomized rats. Brain Res 665:201–212
211. **Dallman MF, Strack AM, Akana SF, Bradbury MJ, Hanson ES, Scribner KA, Smith M** 1993 Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. Front Neuroendocrinol 14:303–347
212. **Ponsalle P, Srivastva LS, Uht RN, White JD** 1992 Glucocorticoids are required for food deprivation-induced increases in hypothalamic neuropeptide Y expression. J Neuroendocrinol 4:585–591
213. **Hanson ES Levin N, Dallman MF** 1997 Elevated corticosterone is not required for the rapid induction of neuropeptide Y gene expression by an overnight fast. Endocrinology 138:1041–1047
214. **White JD, Olchovsky D, Kershaw M, Berelowitz M** 1990 Increased hypothalamic content of preproneuropeptide-Y messenger ribonucleic acid in streptozotocin-diabetic rats. Endocrinology 126: 765–772
215. **Cusin I, Dryden S, Wang Q, Rohner-Jeanrenaud F, Jeanrenaud B, Williams G** 1995 Effect of sustained physiological hyperinsulinaemia on hypothalamic neuropeptide Y and NPY mRNA levels in the rat. J Neuroendocrinol 7:193–197
216. **Sahu A, Dube MG, Phelps CP, Sninsky CA, Kalra PS, Kalra SP** 1995 Insulin and insulin-like growth factor II suppress neuropeptide Y release from the nerve terminals in the paraventricular nucleus: a putative hypothalamic site for energy homeostasis. Endocrinology 136:5718–5724
217. **Porter JP, Bokil HS** 1997 Effect of intracerebroventricular and intravenous insulin on FOS-immunoreactivity in the rat brain. Neurosci Lett 224:161–164
218. **Kalra SP, Xu B, Dube MG, Moldawer LL, Martin D, Kalra PS** 1998 Leptin and ciliary neurotropic factor (CNTF) inhibit fasting-induced suppression of luteinizing hormone release in rats: role of neuropeptide Y. Neurosci Lett 240:45–49
219. **Xu B, Dube MG, Kalra PS, Farmerie WG, Kaibara A, Moldawer LL, Martin D, Kalra SP** 1998 Anorectic effects of the cytokine, ciliary neurotropic factor (CNTF), are mediated by hypothalamic neuropeptide Y (NPY): comparison with leptin. Endocrinology 139:466–473

220. **Spiegelman BM, Flier JS** 1996 Adipogenesis and obesity: rounding out the big picture. Cell 87:377–389
221. **Bray G, Bouchard C** 1997 Genetics of human obesity: research directions. FASEB J 11:937–945
222. **Sclafani A** 1984 Animal models of obesity: classification and characterization. Int J Obes 8:491–508
223. **Chua Jr SC, Brown AW, Kim J, Hennessey KL, Leibel RL, Hirsch J** 1991 Food deprivation and hypothalamic neuropeptide gene expression: effects of strain background and the diabetes mutation. Mol Brain Res 11:291–299
224. **Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A, Mackellar W, Rosteck Jr PR, Schnener B, Smith D, Tinsley FC, Zhang X-Y, Heiman M** 1995 The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 377:530–532
225. **Wang Q, Bing C, Al-Barazanji K, Mossakowaska DE, Wang XM, McBay DL, Neville WA, Taddayon M, Pickavance L, Dryden S, Thomas ME, McHale MT, Gloyer IS, Wilson S, Buckingham R, Arch JR, Trayhurn P, Williams G** 1997 Interactions between leptin and hypothalamic neuropeptide Y neurons in the control of food intake and energy homeostasis in the rat. Diabetes 46:335–341
226. **Frederich RC, Lollmann B, Hamann A, Napolitano-Rosen A, Kahn BB, Lowell BB, Flier JS** 1995 Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. J Clin Invest 96:1658–1663
227. **Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV** 1996 Leptin: the tale of an obesity gene. Diabetes 45:1455–1462
228. **Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P** 1995 Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546–549
229. **Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F** 1995 Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269:540–543
230. **Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rakinowitz D, Lallone RL, Burley SK, Friedman JM** 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543–546
231. **Coleman DL** 1978 Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia 14:141–148
232. **Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Friedman JM** 1996 Abnormal splicing of the leptin receptor in diabetic mice. Nature 379:632–635
233. **Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI, Morgenstern JP** 1996 Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. Cell 84:491–495
234. **Maffei M, Fei H, Lee GH, Dani C, Leroy P, Zhang Y, Proenca R, Negrel R, Ailhaud G, Friedman JM** 1995 Increased expression in adipocytes of ob RNA in mice with lesions of the hypothalamus and with mutations at the db locus. Proc Natl Acad Sci USA 92:6957–6960
235. **White BD, Martin RJ** 1997 Evidence for a central mechanism of obesity in the Zucker rat: role of neuropeptide Y and leptin. Proc Soc Exp Biol Med 214:222–232
236. **Murakami T, Shima K** 1995 Cloning of rat obese cDNA and its expression in obese rats. Biochem Biophys Res Commun 209:944–952
237. **Frankish HM, Dryden S, Hopkins D, Wang Q, Williams G** 1995 Neuropeptide Y, the hypothalamus, and diabetes: insights into the central control of metabolism. Peptides 16:757–771
238. **Hoebel BG** 1984 Neurotransmitters in the control of feeding and its rewards: monoamines, opiates and brain-gut peptides. Res Publ Assoc Res Nerv Ment Dis 62:15–38
239. **Dube MG, Kalra PS, Crowley WR, Kalra SP** 1995 Evidence of a physiological role for neuropeptide Y in ventromedial hypothalamic lesion-induced hyperphagia. Brain Res 690:275–278
240. **Avrith D, Mogenson GJ** 1978 Reversible hyperphagia and obesity following intracerebral microinjections of colchicine into the ventromedial hypothalamus of the rat. Brain Res 153:99–107
241. **Stricker-Krongrad A, Beck B, Burlet C** 1996 Enhanced feeding response to neuropeptide Y in hypothalamic neuropeptide Y-depleted rats. Eur J Pharmacol 295:27–34
242. **Bergen HT, Mobbs CV** 1996 Ventromedial hypothalamic lesions produced by gold thioglucose do not impair induction of NPY mRNA in the arcuate nucleus by fasting. Brain Res 707:266–271
243. **Ahlskog JE, Hoebel BG** 1973 Overeating and obesity from damage to a noradrenergic system in the rat brain. Science 182:166–169
244. **Kyrkouli SE, Stanley BG, Hutchinson R, Seirafi RD, Leibowitz SF** 1990 Peptide-amine interactions in the hypothalamic paraventricular nucleus: analysis of galanin and neuropeptide Y in relation to feeding. Brain Res 521:185–191
245. **Kyrkouli SE, Stanley BG, Leibowitz SF** 1992 Differential effects of galanin and neuropeptide Y on extracellular norepinephrine levels in the paraventricular hypothalamic nucleus of the rat: a microdialysis study. Life Sci 51:203–210
246. **Dube MG, Horvath TL, Leranth C, Kalra PS, Kalra SP** 1994 Naloxone reduces the feeding evoked by intracerebroventricular galanin injection. Physiol Behav 56:811–813
247. **Koegler FH, Ritter S** 1997 Aqueduct occlusion does not impair feeding induced by either third or fourth ventricle galanin injection. Obes Res 5:262–267
248. **Corwin RL, Robinson JK, Crawley JN** 1993 Galanin antagonists block galanin-induced feeding in the hypothalamus and amygdala of the rat. Eur J Neurosci 5:1528–1533
249. **Corwin RL, Rowe PM, Crawley JN** 1995 Galanin and the galanin antagonist M40 do not change fat intake in a fat-chow choice paradigm in rats. Am J Physiol 269:R511–R518
250. **Bartfai T, Fisone G, Langel U** 1992 Galanin and galanin antagonists: molecular and biochemical perspectives. Trends Pharmacol Sci 13:312–317
251. **Leibowitz SF, Kim T** 1992 Impact of a galanin antagonist on exogenous galanin and natural patterns of fat ingestion. Brain Res 599:148–152
252. **Mitchell V, Habert-Ortoli E, Epelbaum J, Aubert JP, Beauvillain JC** 1997 Semiquantitative distribution of galanin-receptor (GALR1) mRNA-containing cells in the male rat hypothalamus. Neuroendocrinology 66:160–172
253. **Howard AD, Tan C, Shiao L-L, Palyha OC, McKee KK, Weinberg DH, Feighner SD, Cascieri MA, Smith RG, Van der Ploeg LH, Sullivan KA** 1997 Molecular cloning and characterization of a new receptor for galanin. FEBS Lett 405:285–290
254. **Akabayashi A, Koenig JI, Watanabe Y, Alexander JT, Leibowitz SF** 1994 Galanin-containing neurons in the paraventricular nucleus: a neurochemical marker for fat ingestion and body weight gain. Proc Natl Acad Sci USA 91:10375–10379
255. **Smith BK, York DA, Bray GA** 1994 Chronic cerebroventricular galanin does not induce sustained hyperphagia or obesity. Peptides 15:1267–1272
256. **Schwartz MW, Sipols AJ, Grubin CE, Baskin DG** 1993 Differential effect of fasting on hypothalamic expression of genes encoding neuropeptide Y, galanin, and glutamic acid decarboxylase. Brain Res Bull 31:361–367
257. **Beck B, Burlet A, Nicolas JP, Burlet C** 1993 Galanin in the hypothalamus of fed and fasted lean and obese Zucker rats. Brain Res 623:124–130
258. **Brady LS, Smith MA, Gold PW, Herkenham M** 1990 Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. Neuroendocrinology 52:441–447
259. **Jhanwar-Uniyal M, Chua Jr SC** 1993 Critical effects of aging and nutritional state on hypothalamic neuropeptide Y and galanin gene expression in lean and genetically obese Zucker rats. Brain Res Mol Brain Res 19:195–202
260. **Morley JE, Levine AS, Yim GK, Lowy MT** 1983 Opioid modulation of appetite. Neurosci Biobehav Rev 7:281–305
261. **Levine AS, Billington CJ** 1989 Opioids: are they regulators of feeding? Ann NY Acad Sci 575:209–219
262. **Lambert PD, Wilding JP, al-Dokhayel AA, Gilbey SG, Bloom SR** 1993 The effect of central blockade of kappa-opioid receptors on neuropeptide Y-induced feeding in the rat. Brain Res 629:146–148
263. **Cole JL, Leventhal L, Pasternak GW, Bowen WD, Bodnar RJ** 1995 Reductions in body weight following chronic central opioid receptor subtype antagonists during development of dietary obesity in rats. Brain Res 678:168–176
264. **Millan MJ, Morris BJ** 1988 Long-term blockade of mu-opioid re-

ceptors suggests a role in control of ingestive behaviour, body weight and core temperature in the rat. Brain Res 450:247–258
265. **Lambert PD, Wilding JP, al-Dokhayal AA, Gilbey SG, Ghatei MA, Bloom SR** 1994 Nalaxone-induced anorexia increases neuropeptide Y concentrations in the dorsomedial hypothalamus: evidence for neuropeptide Y-opioid interactions in the control of food intake. Peptides 15:657–660
266. **Lambert PD, Wilding JP, al-Dokhayal AA, Bohuon C, Comoy E, Gilbey SG, Bloom SR** 1993 A role for neuropeptide-Y, dynorphin, and noradrenalin in the central control of food intake after food-deprivation. Endocrinology 133:29–32
267. **Bergendahl M, Wiemann JN, Clifton DK, Huhtaniemi I, Steiner RA** 1992 Short-term starvation decreases POMC mRNA but does not alter GnRH mRNA in the brain of adult male rats. Neuroendocrinology 56:913–920
268. **Garcia de Yebenes E, Li S, Fournier A, St-Pierre S, Pelletier G** 1995 Regulation of proopiomelanocortin gene expression by neuropeptide Y in the rat arcuate nucleus. Brain Res 674:112–116
269. **Morley JE, Levine AS, Kneip J** 1981 Muscimol induced feeding: a model to study the hypothalamic regulation of appetite. Life Sci 29:1213–1218
270. **Vaughan JM, Fischer WH, Hoeger C, Rivier J, Vale W** 1989 Characterization of melanin-concentrating hormone from rat hypothalamus. Endocrinology 125:1660–1665
271. **Skofitsch G, Jacobowitz DM, Zamir N** 1985 Immunohistochemical localization of a melanin concentrating hormone-like peptide in the rat brain. Brain Res Bull 15:635–649
272. **Bittencourt JC, Presse F, Arias C, Peto C, Vaughan J, Nahon JL, Vale W, Sawchenko PE** 1992 The melanin-concentrating hormone system of the rat brain: an immuno- and hybridization histochemical characterization. J Comp Neurol 319:218–245
273. **Parkes D, Vale W** 1992 Secretion of melanin-concentrating hormone and neuropeptide-E1 from cultured rat hypothalamic cells. Endocrinology 131:1826–1831
274. **Qu D, Ludwig DS, Gammeltoft S, Piper M, Pelleymounter MA, Cullen MJ, Mathes WF, Przypek R, Kanarek R, Maratos-Flier E** 1996 A role for melanin-concentrating hormone in the central regulation of feeding behaviour. Nature 380:243–247
275. **Jezova D, Bartanusz V, Westergren I, Johansson BB, Rivier J, Vale W, Rivier C** 1992 Rat melanin-concentrating hormone stimulates adrenocorticotropin secretion: evidence for a site of action in brain regions protected by the blood-brain barrier. Endocrinology 130: 1024–1029
276. **Presse F, Sorokovsky I, Max JP, Nicolaidis S, Nahon JL** 1996 Melanin-concentrating hormone is a potent anorectic peptide regulated by food-deprivation and glucopenia in the rat. Neuroscience 71:735–745
277. **Max JP, Nahon JL, Burlet A, Nicolas JP, Burlet C** 1993 Rat feeding behavior is not mediated by central injections of MCH. Ann Endocrinol 54:26
278. **Rossi M, Choi SJ, O'Shea D, Miyoshi T, Ghatei MA, Bloom SR** 1997 Melanin-concentrating hormone acutely stimulates feeding, but chronic administration has no effect on body weight. Endocrinology 138:351–355
279. **Griffond B, Deray A, Nguyen NU, Colard C, Fellmann D** 1995 The synthesis of melanin-concentrating hormone is stimulated by ventromedial hypothalamic lesions in the rat lateral hypothalamus: a time-course study. Neuropeptides 28:267–275
280. **Deray A, Griffond B, Colard C, Jacquemard C, Bugnon C, Fellmann D** 1994 Activation of the rat melanin-concentrating hormone neurons by ventromedial hypothalamic lesions. Neuropeptides 27: 185–194
281. **Stanley BG, Willett III VL, Donias HW, Ha LH, Spears LC** 1993 The lateral hypothalamus: a primary site mediating excitatory amino acid-elicited eating. Brain Res 630:41–49
282. **Stanley BG, Ha LH, Spears LC, Dee II MG** 1993 Lateral hypothalamic injections of glutamate, kainic acid, D,L-α-amino-3-hydroxy-5-methyl-isoxazole propionic acid or N-methyl-D-aspartic acid rapidly elicit intense transient eating in rats. Brain Res 613: 88–95
283. **Horvath TL, Naftolin F, Leranth C** 1992 GABAergic and catecholaminergic innervation of mediobasal hypothalamic beta-endorphin cells projecting to the medial preoptic area. Neuroscience 51:391–399
284. **Blasquez C, Jegou S, Feuilloley M, Rosier A, Vandesande F, Vaudry H** 1994 Visualization of gamma-aminobutyric acid A receptors on proopiomelanocortin-producing neurons in the rat hypothalamus. Endocrinology 135:2759–2764
285. **Jegou S, Blasquez C, Delbende C, Bunel DT, Vaudry H** 1993 Regulation of α-melanocyte-stimulating hormone release from hypothalamic neurons. Ann NY Acad Sci 680:260–278
286. **Lambert N, Grover L** 1995 The mechanism of biphasic GABA responses. Science 269:928–929
287. **Staley KJ, Soldo BL, Proctor WR** 1995 Ionic mechanisms of neuronal excitation by inhibitory GABAA receptors. Science 269:977–981
288. **De Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett II FS, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliff JG** 1998 The hypocritins: hypothalamic-specific peptide with neuroexicitatory activity. Proc Natl Acad Sci USA 95:322–327
289. **Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M** 1998 Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 92:573–585
290. **Trivedi P, Yu H, MacNeil D, Van der Ploeg LHT, Guan X-M** 1998 Distribution of orexin receptor mRNA in the rat brain. FEBS Lett 438:71–75
291. **Horvath TL, Diano S, van den Pol AN,** Hypocretin (orexin)-containing neurons make synaptic contact with arcuate nucleus NPY and POMC-producing cells that express leptin receptors in rodents and primates: novel hypothalamic circuit involved in energy homeostasis. Program of the 28th Annual Meeting of the Society for Neuroscience, Los Angeles, CA, 1998, vol 24:12 (Abstract)
292. **Horvath TL, Diano S, van den Pol AN** 1999 Synaptic interaction between hypocretin (orexin) and neuropeptide Y cells in the rodent and primate hypothalamus: a novel circuit implicated in the metabolic and endocrine regulation. J Neurosci 19:1072–1087
293. **Erickson JC, Clegg KE, Palmister RD** 1996 Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. Nature 381:415–421
294. **Erickson JC, Hollopeter G, Palmiter RD** 1996 Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. Science 274:1704–1707
295. **Vale W, Spiess J, Rivier C, Rivier J** 1981 Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213:1394–1397
296. **Antoni FA** 1986 Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor. Endocr Rev 7:351–378
297. **Jones MT, Gillham B** 1988 Factors involved in the regulation of adrenocorticotropic hormone/beta-lipotropic hormone. Physiol Rev 68:743–818
298. **Dunn AJ, Berridge CW** 1990 Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? Brain Res Rev 15:71–100
299. **Britton DR, Koob GF, Rivier J, Vale W** 1982 Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. Life Sci 31:363–367
300. **Morley JE, Levine AS** 1982 Corticotrophin releasing factor, grooming and ingestive behavior. Life Sci 31:1459–1464
301. **Krahn DD, Gosnell BA, Levine AS, Morley JE,** Localization of the effects of corticotropin-releasing factor on feeding. Program of the 14th Annual Meeting of the Society for Neuroscience, 1984, vol 10:302 (Abstract)
302. **Perrin MH, Donaldson CJ, Chen R, Lewis KA, Vale WW** 1993 Cloning and functional expression of a rat brain corticotropin releasing factor (CRF) receptor. Endocrinology 133:3058–3061
303. **Chang CP, Pearse II RV, O'Connell S, Rosenfeld MG** 1993 Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. Neuron 11:1187–1195

304. **Potter E, Sutton S, Donaldson C, Chen R, Perrin M, Lewis K, Sawchenko PE, Vale W** 1994 Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. Proc Natl Acad Sci USA 91:8777–8781
305. **Lovenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, Oltersdorf T** 1995 Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc Natl Acad Sci USA 92:836–840
306. **Heinrichs SC, Menzaghi F, Pich EM, Hauger RL, Koob GF** 1993 Corticotropin-releasing factor in the paraventricular nucleus modulates feeding induced by neuropeptide Y. Brain Res 611:18–24
307. **Kwak SP, Young EA, Morano I, Watson SJ, Akil H** 1992 Diurnal corticotropin-releasing hormone mRNA variation in the hypothalamus exhibits a rhythm distinct from that of plasma corticosterone. Neuroendocrinology 55:74–83
308. **Kwak SP, Morano MI, Young EA, Watson SJ, Akil H** 1993 Diurnal CRH mRNA rhythm in the hypothalamus: decreased expression in the evening is not dependent on endogenous glucocorticoids. Neuroendocrinology 57:96–105
309. **Rohner-Jeanrenaud F** 1995 A neuroendocrine reappraisal of the dual-centre hypothesis: its implications for obesity and insulin resistance. In J Obes Relat Metab Disord 19:517–534
310. **Rohner-Jeanrenaud F, Walker CD, Greco-Perotto R, Jeanrenaud B** 1989 Central corticotropin-releasing factor administration prevents the excessive body weight gain of genetically obese (fa/fa) rats. Endocrinology 124:733–739
311. **Kato H, Saito M, Suda M** 1980 Effect of starvation on the circadian adrenocortical rhythm in rats. Endocrinology 106:918–921
312. **Stanley BG, Lanthier D, Chin AS, Leibowitz SF** 1989 Suppression of neuropeptide Y-elicited eating by adrenalectomy or hypophysectomy: reversal with corticosterone. Brain Res 501:32–36
313. **Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, Sutton S, Chan R, Turnbull AV, Lovejoy D, Rivier C, Rivier J, Sawchenko PE, Vale W** 1995 Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. Nature 378:287–292
314. **Spina M, Merlo-Pich E, Chan RK, Basso AM, Rivier J, Vale W, Koob GF** 1996 Appetite-suppressing effects of urocortin, a CRF-related neuropeptide. Science 273:1561–1564
315. **Heinrichs SC, Lapsansky J, Behan DP, Chan RK, Sawchenko PE, Lorang M, Ling N, Vale WW, De Souza EB** 1996 Corticotropin-releasing factor-binding protein ligand inhibitor blunts excessive weight gain in genetically obese Zucker rats and rats during nicotine withdrawal. Proc Natl Acad Sci USA 93:15475–15480
316. **Carraway R, Leeman SE** 1973 The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. J Biol Chem 248: 6854–6861
317. **Carraway R, Leeman SE** 1975 The aminoacid sequence of a hypothalamic peptide, neurotensin. J Biol Chem 250:1907–1911
318. **Hawkins MF** 1986 Central nervous system neurotensin and feeding. Physiol Behav 36:1–8
319. **Hökfelt T, Everitt BJ, Theodorsson-Norheim E, Goldstein M** 1984 Occurrence of neurotensin-like immunoreactivity in subpopulations of hypothalamic, mesencephalic, and medullary catecholamine neurons. J Comp Neurol 222:543–559
320. **Ibata Y, Kawakami F, Fukui K, Obata-Tsuto HL, Tanaka M, Kubo T, Okamura H, Morimoto N, Yanaihara C, Yanaihara N** 1984 Light and electron microscopic immunocytochemistry of neurotensin-like immunoreactive neurons in the rat hypothalamus. Brain Res 302:221–230
321. **Alexander MJ, Miller MA, Dorsa DM, Bullock BP, Melloni Jr RH, Dobner PR, Leeman SE** 1989 Distribution of neurotensin/neuromedin N mRNA in rat forebrain: unexpected abundance in hippocampus and subiculum. Proc Natl Acad Sci USA 86:5202–5206
322. **Stanley BG, Hoebel BG, Leibowitz SF** 1983 Neurotensin: effects of hypothalamic and intravenous injections on eating and drinking in rats. Peptides 4:493–500
323. **Stanley BG, Eppel N, Hoebel BG** 1982 Neurotensin-injected into the paraventricular hypothalamus suppresses feeding in rats. Ann NY Acad Sci 400:425
324. **Wilding JP, Gilbey SG, Bailey CJ, Batt RA, Williams G, Ghatei MA, Bloom SR** 1993 Increased neuropeptide-Y messenger ribonucleic acid (mRNA) and decreased neurotensin mRNA in the hypothalamus of the obese (ob/ob) mouse. Endocrinology 132: 1939–1944
325. **Beck B, Nicolas JP, Burlet C** 1995 Neurotensin decreases with fasting in the ventromedian nucleus of obese Zucker rats. Metabolism 44:972–975
326. **Rovere C, Viale A, Nahon J, Kitabgi P** 1996 Impaired processing of brain proneurotensin and promelanin-concentrating hormone in obese fat/fat mice. Endocrinology 137:2954–2958
327. **Shimizu I, Hirota C, Obhboshi C, Shima K** 1987 Identification and characterization of glucagon-like peptide 1 7–36 amide binding site in the rat brain and lung. FEBS Lett 241:209–212
328. **Fehmann HC, Goke R, Goke B** 1995 Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. Endocr Rev 16:390–410
329. **Larsen PJ, Tang-Christensen M, Holst JJ, Orskov C** 1997 Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brain stem. Neuroscience 77:257–270
330. **Kreymann B, Ghatei MA, Burnet P, Williams G, Kanse S, Diani AR, Bloom SR** 1989 Characterization of glucagon-like peptide-1-(7–36)amide in the hypothalamus. Brain Res 502:325–331
331. **Shughrue PJ, Lane MV, Merchenthaler I** 1996 Glucagon-like peptide-1 receptor (GLP1-R) mRNA in the rat hypothalamus. Endocrinology 137:5159–5162
332. **Jin SL, Han VK, Simmons JG, Towle AC, Lauder JM, Lund PK** 1988 Distribution of glucagon-like peptide I (GLP-I), glucagon, and glicentin in the rat brain: an immunocytochemical study. J Comp Neurol 271:519–532
333. **Alvarez E, Roncero I, Chowen JA, Thorens B, Blazquez E** 1996 Expression of the glucagon-like peptide-1 receptor gene in rat brain. J Neurochem 66:920–927
334. **Lambert PD, Wilding JPH, Ghatei MA, Bloom SR** 1993 A role for GLP-1 (7–36)$NH_2$ in the central control of feeding behavior. Digestion 54:360–361
335. **Turton MD, O'Shea D, Gunn IN, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR** 1996 A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379:69–72
336. **Tang-Christensen M, Larsen PJ, Goke R, Fink-Jensen A, Jessop DS, Moller M, Sheikh SP** 1996 Central administration of GLP-1-(7–36) amide inhibits food and water intake in rats. Am J Physiol 271:R848–R856
337. **Eng J** 1992 Exendin peptides. Mt Sinai J Med 59:147–149
338. **van Dijk G, Thiele TE, Seeley RJ, Woods SC, Bernstein IL** 1997 Glucagon-like peptide-1 and satiety. Nature 385:214
339. **McMahon LR, Wellman PJ,** PVN infusion of GLP-1 (7–36) amide suppresses feeding and drinking but does not induce conditioned taste aversion or alter locomotion in rats. Program of the 27th Annual Meeting of the Society for Neuroscience, New Orleans, LA, 1997, vol 23:1076 (Abstract)
340. **Goldstone AP, Mercer JG, Gunn I, Moar KM, Edwards CM, Rossi M, Howard JK, Rasheed S, Turton MD, Small C, Heath MM, O'Shea D, Steere J, Meeran K, Ghatei MA, Hoggard N, Bloom SR** 1997 Leptin interacts with glucagon-like peptide-1 neurons to reduce food intake and body weight in rodents. FEBS Lett 415:134–138
341. **Scrocchi LA, Brown TJ, MacLusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ** 1996 Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. Nat Med 2:1254–1258
342. **Yen TT, Gill AM, Frigeri LG, Barsh GS, Wolff GL** 1994 Obesity, diabetes, and neoplasia in yellow A(vy)/− mice: ectopic expression of the agouti gene. FASEB J 8:479–488
343. **Siracusa LD** 1994 The agouti gene: turned on to yellow. Trends Genet 10:423–428
344. **Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD** 1997 Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385:165–168
345. **Lu D, Willard D, Patel IR, Kadwell S, Overton L, Kost T, Luther M, Chen W, Woychik RP, Wilkison WO, Cone RD** 1994 Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. Nature 371:799–802

346. **Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, Lee F** 1997 Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88: 131–141
347. **Jacobowitz DM, O'Donohue TL** 1978 $\alpha$-Melanocyte stimulating hormone: immunohistochemical identification and mapping in neurons of the rat brain. Proc Natl Acad Sci USA 75:6300–6304
348. **Joseph SA, Pilcher WH, Bennet-Clarke C** 1983 Immunocytochemical localization of ACTH perikarya in nucleus tractus solitarius: evidence for a second opiocortin neuronal system. Neurosci Lett 38:221–225
349. **Mountjoy KG, Robbins LS, Mortrud MT, Cone RD** 1992 The cloning of a family of genes that encode the melanocortin receptors. Science 257:1248–1251
350. **Gantz I, Konda Y, Tashiro T, Shimoto Y, Miwa H, Munzert G, Watson SJ, DelValle J, Yamada T** 1993 Molecular cloning of a novel melanocortin receptor. J Biol Chem 268:8246–8250
351. **Roselli-Rehfuss L, Mountjoy KG, Robbins LS, Mortrud MT, Low MJ, Tatro JB, Entwistle ML, Simerly RB, Cone RD** 1993 Identification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. Proc Natl Acad Sci USA 90:8856–8860
352. **Gantz I, Miwa H, Konda Y, Shimoto Y, Tashiro T, Watson SJ, DelValle J, Yamada T** 1993 Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. J Biol Chem 268:15174–15179
353. **Griffon N, Mignon V, Facchinetti P, Diaz J, Schwartz JC, Sokoloff P** 1994 Molecular cloning and characterization of the rat fifth melanocortin receptor. Biochem Biophys Res Commun 200:1007–1014
354. **Gantz I, Shimoto Y, Konda Y, Miwa H, Dickinson CJ, Yamada T** 1994 Molecular cloning, expression, and characterization of a fifth melanocortin receptor. Biochem Biophys Res Commun 200:1214–1220
355. **Mountjoy KG, Mortrud MT, Low MJ, Simerly RB, Cone RD** 1994 Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. Mol Endocrinol 8:1298–1308
356. **Tatro JB** 1990 Melanotropin receptors in the brain are differentially distributed and recognize both corticotropin and $\alpha$-melanocyte stimulating hormone. Brain Res 536:124–132
357. **Schioth HB, Mutulis F, Muceniece R, Prusis P, Wikberg JE** 1998 Discovery of novel melanocortin4 receptor selective MSH analogues. Br J Pharmacol 124:75–82
358. **Kask A, Mutulis F, Muceniece R, Pahkla R, Mutule I, Wikberg JE, Rago L, Schioth HB** 1998 Discovery of a novel superpotent and selective melanocortin-4 receptor antagonist (HS024): evaluation *in vitro* and *in vivo*. Endocrinology 139:5006–5014
359. **Kask A, Rago L, Mutulis F, Pahkla R, Wikberg JE, Schioth HB** 1998 Selective antagonist for the melanocortin 4 receptor (HS014) increases food intake in free-feeding rats. Biochem Biophys Res Commun 245:90–93
360. **Skuladottir GV, Jonsson L, Skarpedinsson JO, Mutulis F, Muceniece R, Raine A, Mutule I, Helgason J, Prusis P, Wikberg JE, Schioth HB** 1999 Long-term orexigenic effect of a novel melanocortin 4 receptor selective antagonist. Br J Pharmacol 126:27–34
361. **Kask A, Rägo L, Korrovits P, Wikberg JE, Schioth HB** 1998 Evidence that orexigenic effects of melanocortin 4 receptor antagonists HS014 are mediated by neuropeptide Y. Biochem Biophys Res Commun 248:245–249
362. **Cheung CC, Clifton DK, Steiner RA** 1997 Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus. Endocrinology 138:4489–4492
363. **Thornton JE, Cheung CC, Clifton DK, Steiner RA** 1997 Regulation of hypothalamic proopiomelanocortin mRNA by leptin in ob/ob mice. Endocrinology 138:5063–5066
364. **Schwartz MW, Seeley RJ, Woods SC, Weigle DS, Campfield LA, Burn P, Baskin DG** 1997 Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes 46:2119–2123
365. **Seeley RJ, Yagaloff KA, Fisher SL, Burn P, Thiele TE, van Dijk G, Baskin DG, Schwartz MW** 1997 Melanocortin receptors in leptin effects. Nature 390:349
366. **Boston BA, Blaydon KM, Varnerin J, Cone RD** 1997 Independent and additive effects of central POMC and leptin pathways on murine obesity. Science 278:1641–1644
367. **Broberger C, Johansen J, Johansson C, Schalling M, Hökfelt T** 1998 The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. Proc Natl Acad Sci USA 95:15043–15048
368. **Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, Trayhurn P** 1996 Localization of leptin receptor mRNA and the long form splice variant (Ob/Rb) in mouse hypothalamus and adjacent brain regions by *in situ* hybridization. FEBS Lett 387:113–116
369. **Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, Morgan PJ, Trayhurn P** 1996 Coexpression of leptin receptor and preproneuropeptide Y mRNA in arcuate nucleus of mouse hypothalamus. J Neuroendocrinol 8:733–735
370. **Gloaguen I, Costa P, Demartis A, Lazzaro D, Di Marco A, Graziani R, Paonessa G, Chen F, Rosenblum CI, Van der Ploeg LH, Cortese R, Ciliberto G, Laufer R** 1997 Ciliary neurotropic factor corrects obesity and diabetes associated with leptin deficiency and resistance. Proc Natl Acad Sci USA 94:6456–6461
371. **Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM** 1996 Leptin enters the brain by a saturable system independent of insulin. Peptides 17:305–311
372. **Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, Prunkard DE, Porte Jr D, Woods SC, Seeley RJ, Weigle DS** 1996 Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 45:531–535
373. **Vasselli JR, Chu K, Maggio CA,** Differential effects of subcutaneous injections of leptin on feeding and body weight in genetically obese, lean and Sprague-Dawley rats. Program of the Annual Meeting of the Society for the Study of Ingestive Behavior, Baltimore, MD, 1997, p 40
374. **Chen G, Koyama K, Yuan X, Lee Y, Zhou YT, O'Doherty R, Newgard CB, Unger RH** 1996 Disappearance of body fat in normal rats induced by adenovirus-mediated leptin gene therapy. Proc Natl Acad Sci USA 93:14795–14799
375. **Muzzin P, Eisensmith RC, Copeland KC, Woo SL** 1996 Correction of obesity and diabetes in genetically obese mice by leptin gene therapy. Proc Natl Acad Sci USA 93:14804–14808
376. **Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton DA, Friedman JM** 1997 Physiological response to long-term peripheral and central infusion in lean and obese mice. Proc Natl Head Sci USA 94:8878–8883
377. **Woods AJ, Stock MJ** 1996 Leptin activation in hypothalamus. Nature 381:745
378. **Van Dijk G, Thiele TE, Donahey JC, Campfield LA, Smith FJ, Burn P, Bernstein IL, Woods SC, Seeley RJ** 1996 Central infusions of leptin and GLP-1-(7–36) amide differentially stimulate c-FLI in the rat brain. Am J Physiol 271:R1096–1100
379. **Håkansson ML, Brown H, Ghilardi N, Skoda RC, Meister B** 1998 Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. J Neurosci 18:559–572
380. **Håkansson M-L, Hulting A-L, Meister B** 1996 Expression of leptin receptor mRNA in the hypothalamic arcuate nucleus–relationship with NPY neurones. Neuroreport 7:3087–3092
381. **Huang XF, Koutcherou I, Lin S, Wang HQ, Storlien L** 1996 Localization of leptin receptor mRNA expression in mouse brain. Neuroreport 7:2635–2638
382. **Lee J, Morris MJ** 1998 Modulation of neuropeptide Y overflow by leptin in the rat hypothalamus, cerebral cortex and medulla. Neuroreport 9:1575–1580
383. **Beck B, Kozak R, Stricker-Krongrad A, Burlet C** 1998 Neuropeptide Y release in the paraventricular nucleus of Long-Evans rat treated with leptin. Biochem Biophys Res Commun 242:636–639
384. **Smith FJ, Campfield LA, Moschera JA, Bailon PS, Burn P** 1996 Feeding inhibition by neuropeptide Y. Nature 382:307
385. **Sahu A** 1998 Leptin decreases food intake induced by galanin (GAL), melanin concentrating hormone (MCH) and neuropeptide Y (NPY) in the rat. Program of the 80th Annual Meeting of The Endocrine Society, New Orleans, LA, 1998 (Abstract P3–253), p 439
386. **Mercer J, Moar KM, Hoggard N** 1998 Localization of leptin re-

ceptor (Ob-R) messenger ribonucleic acid in the rodent brain. Endocrinology 139:29–34
387. **Sahu A** 1998 Evidence suggesting that galanin (GAL), melanin-concentrating hormone (MCH), neurotensin (NT), proopiomelanocortin (POMC) and neuropeptide Y (NPY) are targets of leptin signaling in the hypothalamus. Endocrinology 139:795–798
388. **Satoh N, Ogawa Y, Katsuura G, Tsuiji T, Masuzaki H, Hiroaka J, Kazaki T, Tamoki M, Hyashi M, Yoshimasa Y, Nishin S, Nosoda K, Nakao K** 1997 Pathophysiological significance of the obese gene product, leptin, in ventromedial hypothalamus (VMH)-lesioned rats: evidence for loss of its satiety effect in VMH-lesioned rats. Endocrinology 138:947–954
389. **Armstrong S** 1980 A chronometric approach to the study of feeding behavior. Neurosci Behav Rev 4:27–53
390. **Saladin R, De Vos P, Guerre-Millo M, Leturque A, Girard J, Staels B, Auwerx J** 1995 Transient increase in obese gene expression after food intake or insulin administration. Nature 377:527–529
391. **Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS** 1996 Role of leptin in the neuroendocrine response to fasting. Nature 382:250–252
392. **Baskin DG, Seeley RJ, Kuijper JL, Lok S, Weigle DS, Erickson JC, Palmiter RD, Schwartz MW** 1998 Increased expression of mRNA for the long form of the leptin receptor in the hypothalamus is associated with leptin hypersensitivity and fasting. Diabetes 47: 538–543
393. **Bennett PA, Lindell K, Karlsson C, Robinson IC, Carlsson LM, Carlsson B** 1998 Differential expression and regulation of leptin receptor isoforms in the rat brain: effects of fasting and oestrogen. Neuroendocrinology 67:29–36
394. **Lonnqvist F, Arner P, Nordfors L, Schalling M** 1995 Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. Nat Med 1:950–953
395. **Hamilton BS, Paglia D, Kwan AY, Deitel M** 1995 Increased obese mRNA expression in omental fat cells from massively obese humans. Nat Med 1:953–956
396. **Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM** 1995 Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1:1155–1161
397. **Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF** 1996 Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 334:292–295
398. **Kolaczynski JW, Ohannesian JP, Considine RV, Marco CC, Caro JF** 1996 Response of leptin to short-term and prolonged overfeeding in humans. J Clin Endocrinol Metab 81:4162–4165
399. **Sinha MK, Ohannesian JP, Heiman ML, Kriauciunas A, Stephens TW, Magosin S, Marco C, Caro JF** 1996 Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. J Clin Invest 97:1344–1347
400. **Sinha MK, Sturis J, Ohannesian J, Magosin S, Stephens T, Heiman ML, Polonsky KS, Caro JF** 1996 Ultradian oscillations of leptin secretion in humans. Biochem Biophys Res Commun 228: 733–738
401. **Licinio J, Mantzoros C, Negrao AB, Cizza G, Wong ML, Bongiorno PB, Chrousos GP, Karp B, Allen C, Flier JS, Gold PW** 1997 Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nat Med 3:575–579
402. **Grinspoon S, Gulick T, Askari H, Landt M, Lee K, Anderson E, Ma Z, Vignati L, Bowsher R, Herzog D, Klibanski A** 1996 Serum leptin levels in women with anorexia nervosa. J Clin Endocrinol Metab 81:3861–3863
403. **Spanswick D, Smith MA, Groppi VE, Logan SD, Ashford MLJ** 1997 Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. Nature 390:521–525
404. **Glaum SR, Hara M, Bindokas VP, Lee CC, Polonsky KS, Bell GI, Miller RJ** 1996 Leptin, the obese gene product, rapidly modulates synaptic transmission in the hypothalamus. Mol Pharmacol 50: 230–235
405. **Diano S, Kalra SP, Horvath TL** 1998 Leptin receptor immunoreactivity is associated with the Golgi apparatus of hypothalamic neurons and glial cells. J Neuroendocrinol 10:647–650
406. **Tartaglia LA** 1997 The leptin receptor. J Biol Chem 272:6093–6096
407. **Ghilardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH, Skoda RC** 1996 Defective STAT signaling by the leptin receptor in diabetic mice. Proc Natl Acad Sci USA 93:6231–6235
408. **Vaisse C, Halaas JL, Horvath CM, Darnell Jr JE, Stoffel M, Friedman J** 1996 Leptin activation of STAT3 in the hypothalamus of wild type and ob/ob but not db/db mice. Nat Genet 14:95–97
409. **McCowen K, Chow JC, Smith RJ** 1998 Leptin signaling in the hypothalamus of normal rats *in vivo*. Endocrinology 139:4442–4447
410. **Watanabe S, Arai KI** 1996 Roles of the JAK-STAT system in signal transduction via cytokine. Curr Opin Genet Dev 6:587–596
411. **Starr R, Willson TA, Viney EM, Murray LJ, Rayner JR, Jenkins BJ, Gonda TJ, Alexander WS, Metcalf D, Nicola NA, Hilton DJ** 1997 A family of cytokine-inducible inhibitors of signalling. Nature 387:917–921
412. **Bjorback C, Elmquist JK, Frantz JD, Shoelson SE, Flier JS** 1998 Identification of SOCS-3 as a potential mediator of central leptin resistance. Mol Cell 1:619–625



## Erratum

The title of the paper "The Thyrotropin (TSH)-Releasing Hormone Receptor: Interaction with TSH and Autoantibodies (B. Rapoport *et al.*, *Endocrine Reviews* 19:673–716, 1998) was incorrect. The correct title, "The Thyrotropin (TSH) Receptor: Interaction with TSH and Autoantibodies," was inadvertently altered during typesetting. We apologize for the error.

The correct version of this title page appears on the facing page.