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Neuropeptide Y

A Physiological Orexigen Modulated by the Feedback Action of Ghrelin and Leptin

Satya P. Kalra and Pushpa S. Kalra

Department of Neuroscience and Department of Physiology & Functional Genomics, University of Florida McKnight Brain Institute, Gainesville, FL

Neuropeptide Y (NPY), a 36-amino-acid neuropeptide is the most potent physiological appetite transducer known. Episodic NPY neurosecretion in hypothalamic target sites is temporally linked with onset of the daily feeding pattern. Upregulation of NPY signaling in the arcuate nucleus–paraventricular nucleus (ARC-PVN) neural axis is responsible for the hyperphagia evoked by dieting, fasting, hormonal and genetic factors, and disruption in intrahypothalamic signaling. Clusters of NPY-producing neurons in the ARC that coexpress γ-amino butyric acid and agouti-related peptide, and those in the brain stem (BS) that coexpress catecholamines and galanin, participate in disparate manners to regulate appetitive behavior. NPY receptors, Y1, Y2, and Y5, expressed by various components of the NPY network, mediate NPY-induced feeding. Imbalance in NPY signaling due either to high or low abundance of NPY at target sites elicits hyperphagia leading to increased fat accretion and obesity. Recent studies show that intermittent, feedback action of opposing afferent hormonal signals—leptin from adipose tissue and ghrelin from stomach—regulate the episodic secretion of orexigenic NPY in the PVN-ARC. Apparently, the hypothalamic NPY network is the primary common pathway intimately involved in genesis of appetite-stimulating impulses.

Key Words: Neuropeptide Y; hypothalamus; appetite; leptin; ghrelin; hypothalamic network.

Introduction

All living organisms require food (a nutritional supply) for growth and maintenance. Feeding in vertebrates is a periodic behavior. Episodes of feeding behavior alternate with periods of nonfeeding even in the presence of an *ad libitum* supply of food. The impetus to eat, i.e., expression of appetite, is chemically coded in the brain. Orexigenic neural pathways drive the organism to a source of food in a timely fashion and initiate eating on their own and also by simultaneously repressing anorexigenic signaling *(1,2)*. A variety of external and internal factors modulate expression of periodic appetite on a daily basis. It is apparent now that derangements in this interplay produced either externally by varied quality of food and rates of energy disposal, or internally by shifts in hormonal, metabolic and genetic factors, disrupt the hypothalamic integration of energy balance, the cornerstone in rigidly guarding body weight around a set point *(1–7)*.

The origin of this newer insight, that the appetite regulating network (ARN) resides in the hypothalamus, can be traced to clinical observations at the turn of the 20th century of an association between hypothalamic tumors and obesity and to experimental observations in rats of relentless phagia and obesity in response to lesions in the ventromedial hypothalamus (VMH) and inanition in response to lesions in the lateral hypothalamus (LH). Subsequently, indirect evidence supported the concept of dual hypothalamic centers in which neural signals from the VMH restrained activation of the "hunger" network in the LH *(8–11)*. Although this tenet of reciprocal interplay in governing weight homeostasis occupied the attention for over 40 yr, it lost ground as critical experimental testing failed to identify specific neurochemical substrates in these loci capable of reliably affecting ingestive behavior *(8)*.

The report in 1984 that intraventricular administration of neuropeptide Y (NPY) stimulated feeding *(12)*, and NPYergic pathways, emanating from the arcuate nucleus (ARC) and terminating in the paraventricular nucleus (PVN) of the hypothalamus, participated in a physiologically relevant manner in regulating ingestive behavior, laid the foundation of the current concept that appetite expression is chemically coded in a discrete ARC-PVN neuroaxis (Fig. 1; *1,13–15*). These revelations sparked multidisciplinary research to gain a deeper understanding of the hypothalamic control of appetite. Two additional circuits, the anorexigenic one also emanating in the ARC and the modulatory one emanating in the VMH and LH, were identified in the ensuing decade *(1,4, 16–19)*. It is now evident that each of these components of

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Author to whom all correspondence and reprint requests should be addressed: Satya P. Kalra, PhD, Department of Neuroscience, University of Florida McKnight Brain Institute, P.O. Box 100244, Gainesville, FL 32610-0244. E-mail: skalra@ufbi.ufl.edu

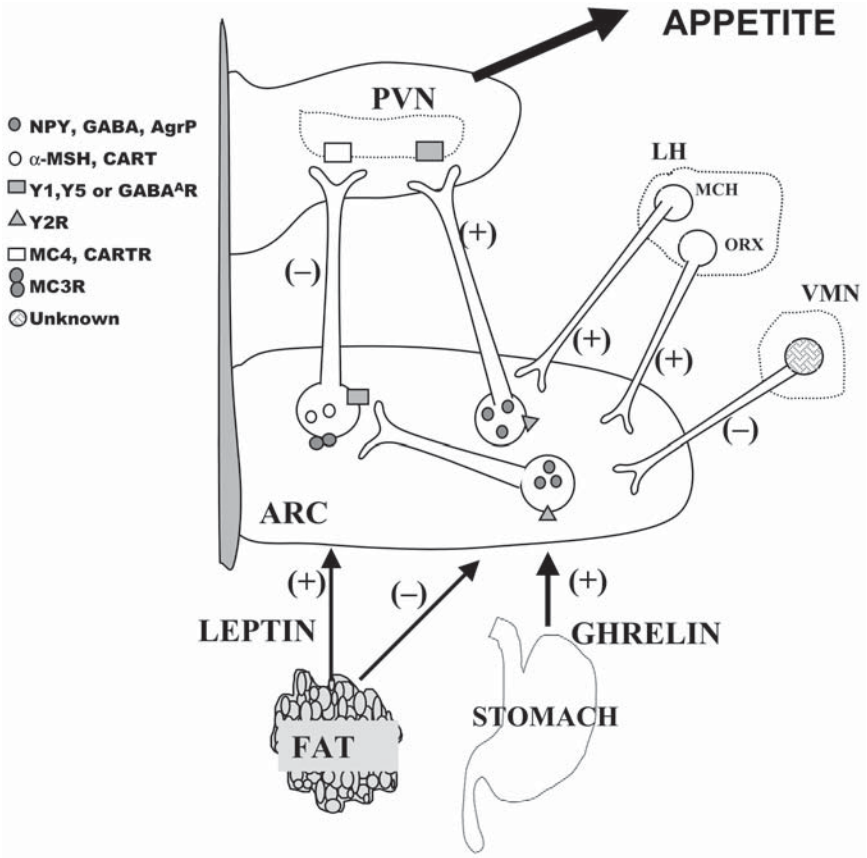

Fig. 1. Diagrammatic representation of interconnections of the hypothalamic appetite-regulating circuitry modulated by the opposing feedback actions of leptin from fat tissue and ghrelin from stomach. NPY neurons coexpressing GABA and AgrP in the arcuate nucleus (ARC) project into the paraventricular nucleus (PVN) where NPY, AgrP, and GABA release stimulate appetite by activating Y1/Y5, MC4, and $GABA^A$ receptors (R), respectively. In addition, NPY, AgrP, and GABA released in the ARC suppress the restraint of ARC POMC neurons coexpressing α-MSH and CART on appetite by activating Y1/Y5, MC3, and $GABA^AR$. Thus, a two-prong action of NPY neurons, one by direct activation of target sites in the PVN and, two by indirectly curtailing the melanocortin restraint of the ARC POMC neurons, results in appetite stimulation. Whereas inhibition of appetite by leptin involves a restraint on NPYergic signaling and stimulation of melanocortin signaling, stimulation of appetite by ghrelin is solely due to enhanced orexigenic NPY network. MCH and ORX produced in the lateral hypothalamus (LH) stimulate feeding by involving NPY network, but the phenotype of neurons that exert an inhibitory influence on appetite-regulating circuitry is unknown.

the ARN is functionally linked with the NPY pathway in the ARC-PVN axis (Fig. 1; *1,16,20–22*). The objective of this article is to review the dynamics of the orexigenic NPY network in eliciting the periodic appetitive drive under the feedback influence of peripheral hormonal signals, leptin from adipocytes and ghrelin from stomach *(1–3,23,24)*.

NPY and Appetite

Effects on Feeding

NPY is the most potent appetite stimulating molecule produced in the brain *(1,2,15)*. Administration of this 36-amino-acid neuropeptide, belonging to the pancreatic polypeptide family, has been shown to stimulate feeding in all vertebrates species tested, including subhuman primates *(1, 2,15,26,27)*. Stimulation of feeding is rapid and is sustained for hours. NPY also augments ongoing feeding in a dose-dependent manner *(14,28)*. Continuous intraventricular NPY infusion evoked feeding episodes that lasted for a considerable post-infusion period *(29)*. NPY-induced hyperphagia promoted weight gain, accretion of fat, and hyperinsulinemia, a phenotype resembling the obesity syndrome. There was no evidence of tolerance to NPY-induced increased ingestive behavior in rats *(29,30)*. Although experimental evidence suggested multiple sites of NPY action in the hypothalamus, coinciding with the wide distribution pattern of NPY receptors *(27,31–33)*, NPY release was found to be augmented selectively in the PVN in association with fasting-induced hyperphagia and onset of normal feeding *(34–36)*. It is highly likely that stimulation of appetite is medi-

ated by the high abundance of NPY receptors in the PVN, especially in the magnocellular PVN (mPVN), and sparse abundance in neighboring sites (Fig. 1; *37–41*).

That NPY is physiologically relevant in information relay for food acquisition on a daily basis is evidenced by several of experimental findings. NPY secretion is increased in the PVN in association with increased hunger produced by fasting or anticipation of food availability in rats maintained on a restricted daily feeding regimen *(26,34–36)*. That the high amplitude and frequency of NPY release triggers the drive for energy repletion is indicated by observations that neutralization of NPY with NPY antibodies or blockade of NPY receptors pharmacologically extinguished the fasting and dark-phase appetitive drive *(1,26,34–46)*. Thus, the close temporal relationship between heightened expression of appetite and NPY hypersecretion in the PVN validates the hypothesis that NPY is one of the key physiological messenger molecules in the hypothalamus that encodes acquisition of daily intake *(12,13,26)*.

Interestingly, enhanced NPY synthesis in the ARC, as reflected by increased gene expression, precedes the increased storage of NPY in PVN nerve terminals for timely release *(34,47–49)*. Furthermore, whereas decrease in storage and release in the PVN closely follows food consumption and ensuing satiety, NPY synthesis in the ARC continues for a considerable period. These lines of evidence are consistent with the view that changing patterns of NPY neurosecretion in the hypothalamus are temporally linked to both the expression and extinction of appetite.

NPY, Eating Disorders, and Obesity

High Abundance and Lack of Tolerance

The role of the brain in clinical manifestation of eating disorders and behavior has been at the forefront of research for the past two decades. The current view holds that alteration in patterns of eating behavior is an important etiological factor for the development of obesity. In light of these implications, our observations that NPY evokes a discontinuous feeding pattern and the magnitude of feeding episodes is dependent on the quantity of NPY receptor activation, which, in turn, impacts feeding duration and local eating rate (g/min) are seemingly important *(29)*. In addition, experimental demonstrations that extremely low level of NPY receptor activation can sustain the motivation to acquire food for a considerable period of time even in the absence of readily available food, and the observation of a slow decline in the rate of food consumption after cessation of NPY receptor activation, are suggestive of a role of NPY in increased periodic eating in obese patients. We propose that hyperphagia and increased bouts of eating behavior in obese patients may be due to increase in the rate of episodic NPY secretion. Because there is no evidence of tolerance to NPY receptor activation, it is likely that environmental factors that stimulate NPY secretion in the PVN and neighboring sites have the potential to provoke increased energy intake leading to accelerated rate of storage of excess energy as fat depot.

Obesity due to genetic factors is invariably associated with increased NPY signaling in rodents *(1,6)*. Administration of either NPY receptor antagonists or cytokines capable of suppressing NPY synthesis suppress food intake and normalize weight in obese rodents *(37–46,49–51)*. Even NPY-knockout mice, which do not normally show any change in daily food consumption, are incapable of displaying hyperphagia normally evoked by compete lack of leptin, as in ob/ob mice, or diminution in leptin feedback resulting from fasting or experimentally induced diabetes *(52–56)*. Thus, an intact NPY network in the hypothalamus is critical for induction of hyperphagia and obesity.

It is well known that estrogens decrease weight gain and food intake *(1)*. We showed that estrogen treatment decreased NPY levels and release in the PVN in association with diminished intake and reduced weight gain *(57)*. The sites of action of estrogen in suppressing food intake apparently reside in the ARC, where NPY neurons coexpress the estrogen receptor, and in the PVN, where afferents of PVN estrogen-concentrating cells, may suppress NPY storage and release locally *(1,57–59)*.

Low Abundance and Enhanced Receptor Sensitivity

Another unanticipated and intriguing way NPY signaling in the ARC-PVN participates in hyperphagia culminating in obesity is the development of enhanced sensitivity to NPY in response to diminished NPY release in both ARC and PVN *(60–66)*. Experiments designed to interrupt neural input to the ARC-PVN by either indiscriminate destruction of large amounts of neural tissue in the VMH, interruption of neural signaling discretely in the VMN pharmacologically, or transection of neural input from the brain stem by knife cuts resulted in marked decrease in NPY synthesis in the ARC and release in the PVN. Despite these deficits in orexigenic neurochemical signals, these rats displayed hyperphagia leading to obesity. Further investigations revealed that low abundance of NPY rearranged the appetite-generating signaling in a fashion that resulted in upregulation of NPY receptors. Higher abundance of NPY receptor rendered these animals hypersensitive to NPY such that extremely low doses of NPY that are normally ineffective, evoked near maximal stimulation of feeding. NPY Y1R antagonist and passive immunoneutralization with NPY antibodies attenuated the exaggerated feeding in these rats *(62,64)*. Furthermore, it was found that an increase in NPY Y1 receptor abundance accounted for enhanced sensitivity, which, in part, resulted from a loss of melanocortin restraint *(62,66)*, and, in part, from an ineffectiveness of peripheral hormonal signal, leptin, to regulate NPY signaling *(64–67)*. Another factor that may contribute to the development of hyperphagia in these rats is augmented availability of NPY for action outside the PVN, as NPY gene

expression was found to increase in the neighboring dorsomedial hypothalamus *(68)*.

Obviously, imbalance in local NPYergic signaling due to either high or low abundance elicits disparate neurochemical sequelae that evoke hyperphagia and obesity.

Mechanism of NPY Action

Differential Roles of NPY–Producing Perikarya in the Brain Stem and ARC in the Hypothalamic Integration of Appetitive Drive

A series of studies, undertaken to characterize the nature of participation of varied NPY subpopulations of neurons innervating hypothalamic sites, showed that both the extrahypothalamic subpopulation in the brain stem (BS) and the intrahypothalamic subpopulation in the ARC and possibly the DMN, engage disparate neurochemical signaling modalities in the daily management of feeding behavior *(60,69)*. NPY neurons in the BS coexpress norepinephrine, epinephrine, and galanin, each of which can stimulate feeding on its own, and synergistically with NPY *(69–71)*. Elimination of BS projections produced a deficit in these coexpressed neurotransmitters in the hypothalamus, which was associated with increased sensitivity to NPY and hyperphagia that progressively led to increase in the body weight (*60,61*; unpublished observations). Thus, we showed that NPY of extrahypothalamic source, along with the coexpressed orexigenic signals, are engaged in the genesis and consolidation of neural stimuli that initiate and regulate nocturnal appetite.

On the other hand, NPY-producing perikarya in the ARC coexpress AgrP and GABA and project to those same feeding relevant sites (Fig. 1; *1,72,73*). Synergism at postsynaptic targets between NPY and that coexpressed orexigenic signals involving distinct receptors has been documented *(74,75)*. This interplay together with the observed parallel shifts in gene expression of these three messengers in response to fasting *(49,50,76–78)*, similar mediatory responses to anorexigenic leptin and orexigenic ghrelin from the periphery *(1,3)*, and the observed dynamic patterns of NPY synthesis and release in association with onset of enhanced appetite *(34,36)*, strongly corroborate our early proposal that the ARC NPY perikarya and their projections to the PVN and neighboring sites constitute the final common pathway in the daily management of appetitive behavior. (Fig. 1)

Morphological and Functional Relationship of the NPY ARC–PVN Network with Varied Intrahypothalamic Orexigenic and Anorexigenic Pathways

Extensive morphological studies have revealed an intricate link between the ARC NPY neurons and other neurons producing orexigens, such as galanin (GAL), orexin (ORX), melanin concentrating hormone (MCH), and β-endorphin and neurons producing anorexigens such as melanocyte stimulating hormone (α-MSH), cocaine and amphetamine regulating transcript (CART), in various hypothalamic sites *(1,79–81)*. Experimental evidence accumulated over several years support a role of information transfer along the NPY ♦ GAL ♦ β-END pathway in stimulation of feeding by NPY *(1)*. On the other hand, a restraining influence of NPY and GABA on ARC proopiomelanocortin (POMC) neurons resulting in diminution of α-MSH and, possibly, CART release in the PVN, may assist in stimulation of appetite by NPY (Fig. 1; *1,3,82–89*). Thus, a three-prong action, direct stimulation of PVN NPY receptors by NPY, increased release of the orexigenic opioid, β-END via stimulation by GAL and GAL itself, along with a simultaneous restraint on anorexigenic α-MSH and CART signaling, is likely to initiate and sustain robust dark-phase appetite in rodents. Pharmacological and morphological evidence also showed that the orexigenic effects of ORX and MCH occur via stimulation of NPY release by action either at the ARC perikaryal level or at the PVN nerve terminal level *(16,20–22)*. It is likely that hormonal and neural afferents from the periphery transmit the energy status in the body to the NPY ARC-PVN axis via orexigenic networks in the LH.

Distinct Roles of Various NPY Receptors

Of the two putative receptors, Y1R and Y5R, implicated in mediation of the postsynaptic orexigenic effects of NPY, Y1R seems to be the primary one *(27,32,37–46,90,91)*. Germ line deletion of either Y1 or Y5R results in late onset obesity. Selective Y1R antagonists block feeding in Y5R-knockout mice, and, similarly, Y5R antagonist attenuate feeding in Y1R-knockout mice, suggest that both receptors may participate in stimulation of appetite *(92–95)*. However, experimental evidence that fasting selectively increased Y1R gene expression, and suppression of Y1R gene receptor by anorexic cytokines, leptin and ciliary neurotrophic factor, in association with the fasting-induced suppression of appetite, are consistent with our original proposal *(49,50,90)*, that signal transmission by Y1R is crucial in the genesis of appetite-stimulating impulses. Further evidence derived from c-fos activation showed that postsynaptic Y1/Y5 R expressing targets are resident primarily in the mPVN, where, recently, Y1 and Y5 have also been visualized (Fig. 1; *37–39*).

Although the existence of presynaptic Y2R autoreceptors have been known for a long time, their role in NPY-induced feeding has not been clearly defined *(27,32,35,91)*. We observed that intraventricular NPY infusion suppressed NPY gene expression in the ARC neurons *(95)*. This observation coupled with the demonstration of Y2R expression by ARC NPY neurons suggests autoregulation of NPY secretion locally in the ARC via Y2R receptors (Fig. 1; *91*). Indeed, in conditional hypothalamic Y2R knockout mice, NPY and AgrP expression were increased along with upregulation of POMC and CART gene expression in the ARC *(96)*. These observations are in agreement with the notion that NPY released in the ARC regulates NPY gene expression through presynaptic Y2R, and the resultant diminution in

NPY release in the ARC allows increased POMC and CART expression *(1)*, thus causing dysregulation in hypothalamic integration of weight control.

Regulation of NPY Secretion

Daily Pattern of NPY Synthesis and Release

The ARC NPY neurons secrete NPY in an episodic manner selectively in the PVN. During periods of nonfeeding in the light phase, NPY is released in the form of low-amplitude episodes. However, at the onset of the dark-phase NPY hypersecretion, characterized by high-amplitude episodic discharge at accelerated pace, elicits a corresponding increase in feeding episodes *(34,36)*. As NPY secretion subsides, feeding ceases. Increased synthesis in the ARC and storage of NPY in the PVN nerve terminals precedes the anticipated trigger of NPY hypersecretion in the PVN *(34, 47,48)*. This temporal lag between augmented NPY stores available for release and the trigger of NPY release is likely the function of a neural clock that times the antecedent increase in NPY synthesis and storage and, subsequently, release in the PVN *(1)*.

Regulation of NPY Secretion by Afferent Hormonal Signals Leptin and Ghrelin

Information amassed during the past decade has shown that reciprocal action of afferent hormonal signals, leptin produced by adipocytes and ghrelin produced by stomach, regulates NPY secretion for the daily management of food intake (Fig. 1; *1,3,23,24,97–100*). Leptin is an anorexigenic signal that restrains NPY neurosecretion primarily by suppressing NPY synthesis in the ARC. Furthermore, it upregulates melanocortin signaling on its own and secondary to diminishing the restraint exerted by NPY *(1,23,101,102)*. Leptin is secreted in episodic fashion *(103,104)*. Analysis of the daily pattern of circulating leptin levels indicated that leptin hypersecretion follows night-time feeding, a signal seemingly responsible for gradually terminating the night-time feeding. Thereafter, leptin secretion slowly decreases to a low before the dark-phase onset of feeding *(48,101)*. Our studies, and those of others, suggested that this low level of leptin restraint facilitates expression of orexigenic pathways leading to the appetitive drive *(3,48,101)*. This mode of leptin involvement through a restraint on orexigenic pathways is supported by the fact that absence of either leptin or leptin receptor in the hypothalamus resulted in hyperphagia and morbid obesity and restoration of optimal levels of leptin normalized food intake and weight *(23,101)*. Fasting or food restriction diminished leptin secretion resulting in enhanced appetite due to increased NPY secretion and the curtailed leptin restraint on melanocortin signaling *(1,3,34–36)*. Increased expression of leptin locally in the hypothalamus by gene therapy suppressed appetite and weight gain by a sustained restraint on NPY and enhanced melanocortin signalings *(105–107)*.

Ghrelin has recently been shown to stimulate food intake and promote adiposity *(97–100,108)*. Ghrelin is considered to be a physiological afferent hormonal signal in daily patterning of ingestive behavior *(3)*. Stimulation of appetite by ghrelin apparently results from increased NPY release and Y1R mediation in the PVN *(99,100,109,110)*. Ghrelin is secreted in pulsatile fashion, and secretion is augmented prior to mealtime *(111,112)*; fasting increased episodic secretion of ghrelin concomitant with diminished leptin secretion *(111)*. This reciprocal intermittent afferent regulatory signaling of anorexigenic leptin and orexigenic ghrelin at the level of the NPY network in the hypothalamus is believed to play a major role not only in synchronizing appetite generating impulses but also in terminating sensations of hunger in rodents and humans *(1,3)*.

Concluding Remarks

It is clear that appetite-stimulating impulses emanate within the hypothalamus as a consequence of a complex interplay between environmental factors and the ARN in the brain. Within the ARC-PVN axis, the NPY network is apparently the common final pathway responsible for stimulation of appetite. NPY is the only known neurotransmitter whose secretion pattern at target sites is correlated with the onset of feeding and hyperphagia induced by fasting and dieting. NPY secretion is regulated by reciprocal feedback action of orexigenic ghrelin and anorexigenic leptin. The outstanding features of the NPY network operation are that it is intimately involved in modulation of the output of a host of orexigenic and anorexigenic signaling modalities within the ARN. Various disruptions that elicit low or high abundance of NPY at target sites dysregulate the hypothalamic homeostasis causing unregulated phagia and abnormal weight gain. Seemingly, ARC NPY neurons conduct the appetite regulating network orchestra in the hypothalamic integration of energy homeostasis.

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