Frontiers in Neuroendocrinology 32 (2011) 398–415

Contents lists available at ScienceDirect

Frontiers in Neuroendocrinology

journal homepage: www.elsevier.com/locate/yfrne

Review

The role of NPY in hypothalamic mediated food intake

Rebecca E. Mercer [a], Melissa J.S. Chee [b], William F. Colmers [c,*]

[a] Department of Medical Genetics, University of Alberta, Edmonton, AB, Canada T6G 2H7
[b] Division of Endocrinology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, United States
[c] Department of Pharmacology, University of Alberta, Edmonton, AB, Canada T6G 2H7

ARTICLE INFO

Article history:
Available online 25 June 2011

Keywords:
Neuropeptide Y
Animal models
Food intake
Energy balance
Compensation
Redundancy
Integration
Hypothalamus

ABSTRACT

Neuropeptide Y (NPY) is a highly conserved neuropeptide with orexigenic actions in discrete hypothalamic nuclei that plays a role in regulating energy homeostasis. NPY signals via a family of high affinity receptors that mediate the widespread actions of NPY in all hypothalamic nuclei. These actions are also subject to tight, intricate regulation by numerous peripheral and central energy balance signals. The NPY system is embedded within a densely-redundant network designed to ensure stable energy homeostasis. This redundancy may underlie compensation for the loss of NPY or its receptors in germline knockouts, explaining why conventional knockouts of NPY or its receptors rarely yield a marked phenotypic change. We discuss insights into the hypothalamic role of NPY from studies of its physiological actions, responses to genetic manipulations and interactions with other energy balance signals. We conclude that numerous approaches must be employed to effectively study different aspects of NPY action.

1. Introduction

Obesity, defined as a state of increased adiposity resulting from chronic nutrient excess, has reached epidemic levels in modern society, and is currently one of the greatest burdens to health care from both the population and economic standpoints. In order to maintain normal energy balance, the body relies on homeostatic processes to detect nutrient and energy levels and then integrates this information to coordinate food intake and energy expenditure accordingly. The hypothalamus, a specialized region of the brain, is one of the main nutrient sensors. It is within the hypothalamus that multiple nutrient related signals of peripheral and central origin converge and become integrated [214]. Therefore, understanding how the hypothalamus coordinates information to control energy balance is critical for understanding the pathology of obesity, as well as for designing treatments to relieve the burden of this health condition.

Neurons in several distinct hypothalamic nuclei have been linked to energy homeostasis, including ones in the arcuate nucleus (ARC) that project to the paraventricular nucleus (PVN), ventromedial nucleus (VMN), lateral hypothalamic area (LHA), and dorsomedial nucleus (DMN). The importance of the hypothalamus in the regulation of energy balance was already established in the 1940s with the pioneering work by Hetherington and Ranson [124] demonstrating that bilateral lesions to different hypothalamic areas led to dramatic body weight gain or loss. The energy status of the body is conveyed within the hypothalamus by several classes of chemical messengers, including amino acid transmitters [14,56], biogenic amines [28,290,305], cytokines [3,42], cannabinoids [300], and neuropeptides [48]. Injection of these chemical messengers into discrete hypothalamic nuclei results in specific types of energy homeostatic responses. While all have effects, the most profound and prolonged responses tend to be mediated by the neuropeptides.

Neuropeptide Y (NPY) is one of the earliest-recognized and most potent orexigenic neuropeptides acting in the hypothalamus. NPY is a 36 amino acid peptide originally isolated from the porcine brain in 1982 [281] and has since been identified in many invertebrate and vertebrate species [29,225]. The full NPY gene sequence has been identified in at least nine species, and sequence comparisons with the human NPY gene confirm a remarkable degree of evolutionary conservation, with the least similar ortholog, in the

Abbreviations: 2-AG, 2-arachidonoylglycerol; AAV, adeno associated virus; AEA, N-arachidonoylethanolamide/anandamide; AgRP, agouti-related peptide; α-MSH, alpha melanocyte stimulating hormone; ARC, arcuate nucleus; BBB, blood–brain barrier; CART, cocaine and amphetamine regulated transcript; CB, cannabinoid receptors; DIO, diet induced obesity; DIO–R, DIO–resistant; DIO–S, DIO–sensitive; dKO, double knockout; DMN, dorsomedial hypothalamic nucleus; DMV, dorsal motor nucleus of the vagus; DOX, doxycycline; DT, diphtheria toxin; f, fornix; GABA, γ-aminobutyric acid; GAL, galanin; GALP, galanin-like peptide; ICV, intracerebroventricular; LHA, lateral hypothalamic area; MCH, melanin concentrating hormone; MCR, melanocortin receptors; NPY, neuropeptide Y; NPY-OE, NPY overexpressing; OLETF, Otsuka Long-Evans Tokushima Fatty; PFA, perifornical area; POMC, pro-opiomelanocortin; PP, pancreatic polypeptide; PVN, paraventricular nucleus; PYY, peptide YY RNAi, RNA interference; VMN, ventromedial nucleus.

* Corresponding author. Address: 9-36 Medical Science Building, Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7. Fax: +1 780 492 4325.
E-mail address: william.colmers@ualberta.ca (W.F. Colmers).

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doi:10.1016/j.yfrne.2011.06.001

zebrafish, still showing over 75% similarity at the nucleotide level (Table 1). NPY conservation is even more striking at the peptide level, with most mammalian sequences being identical or nearly identical bearing only conservative amino acid substitutions [174]. Non mammalian species also show a striking degree of NPY sequence similarity. For example, the chicken and *Torpedo* NPY sequences differ from the human sequence at only one and three positions, respectively [29]. The strong evolutionary conservation of NPY suggests a vital role for NPY in physiology. Aside from its role in the stimulation of food intake, NPY has been implicated in other physiological processes including locomotion [121], learning and memory [96], anxiety [109], epilepsy [158,299], circadian rhythm [265], and cardiovascular function [135].

Over the last three decades since the discovery of NPY, various experimental techniques ranging from genetic manipulation of NPY or its receptors to electrophysiological investigations have been employed to understand the metabolic, behavioural, and mechanistic effect of hypothalamic NPY on energy homeostasis. This review will discuss the orexigenic role of hypothalamic NPY and its effects in specific hypothalamic nuclei, examine the function and modulation of NPY receptors in the regulation of food intake, and highlight the major role of NPY in the integration of both peripheral and central signals regulating energy balance.

2. Evidence for NPY as an orexigenic neuropeptide

Within 2 years after it was first described, NPY was shown to increase food intake. Intracerebroventricular (ICV) injection of NPY stimulated food intake in a dose-dependent manner [55,185]. The specific sites of NPY action in the hypothalamus that elicit a feeding response were identified by intranuclear injection of small volumes of NPY; these sites include the perifornical area (PFA) of the LHA, PVN, and VMN [269]. Moreover, chronic administration of NPY produces a sustained hyperphagia and increased body weight gain leading to obesity [23,323].

In several models of animal obesity, such as in diet-induced obesity (DIO) [114], tubby mice [115], melanocortin receptor 4-knockout mice (MC4R-KO) [156], leptin-deficient *ob/ob* mice [156,310], agouti yellow mice [156], Zucker fatty *fa/fa* rats [253], and brown adipose tissue-deficient mice [292], the levels of NPY gene expression are elevated. The release of NPY is also elevated in the obese state, as shown in the Zucker rat [80], and pharmacological treatments that enhance feeding also increase NPY gene expression [186].

Table 1
Orthologs of the neuropeptide Y gene.

Organism	Scientific name	NCBI[a] accession	Similarity to human NPY (%)		
			Nucleotide	Pro-peptide	Peptide
Human	*Homo sapiens*	NP_000896.1	100	100	100
Chimpanzee	*Pan troglodytes*	XP_001159002.1	99.3	99.0	100
Dog	*Canis lupus*	XP_532492.2	92.1	95.9	100
Cow	*Bos taurus*	NP_001014845.1	91.4	94.9	97.2
Mouse	*Mus musculus*	NP_075945.1	88.0	92.8	100
Rat	*Rattus norvegicus*	NP_036746.1	88.7	92.8	100
Chicken	*Gallus gallus*	NP_990804.1	84.2	83.5	97.2
Frog	*Xenopus laevis*	L11294.1	76.8	78.4	97.2
Zebrafish	*Dania rerio*	NP_571149.1	75.7	67.0	88.9

[a] National Center for Biotechnology Information.

NPY release is enhanced immediately prior to the onset of feeding and gradually decreases as food intake continues [145]. Increases in NPY expression [246] precede hyperphagic behaviour and are directly correlated with conditions of increased energy deprivation [34] and energy demand (i.e. during lactation [268]). Conversely, the actions of NPY, even fasting-induced refeeding [63,173], can be blocked by the pharmacological application of NPY antagonists.

2.1. Production of NPY in the hypothalamus

The main source of NPY within the hypothalamus is from specialized neurons in the ARC [54]. Along with NPY, a great majority of these neurons also co-express the orexigenic agouti-related peptide (AgRP) [39]. In addition to being made in the ARC, NPY can also be produced by hypothalamic DMN neurons, though only transiently during certain states of negative energy balance. Thus, NPY expression is high in DMN neurons in the early postnatal period when growth rates are rapid [110–112], and then DMN NPY expression levels fall rapidly and remain low in adult rodents. However, NPY expression can be induced to high levels in the DMNs of lactating females [51,188,189] and of rodent models of obesity resulting from genetics or diet [27,114,115,156,312]. Taken together, these studies indicate a role for DMN NPY in the maintenance of hyperphagia in conditions of real or perceived energy demand.

Outside of the hypothalamus, NPY expressing neurons have been found throughout the central nervous system, with particularly high levels of NPY expression and immunoreactivity found in brain stem and hippocampal regions [71,295]. While extrahypothalamic NPY certainly impacts the numerous physiologic roles of NPY including energy homeostasis, it remains to be determined how much these sources contribute to the actions of NPY within the hypothalamus. The remainder of this review will focus specifically on NPY production and action within the hypothalamus.

2.2. NPY mediated antagonism of the anorexigenic melanocortin system

In addition to the orexigenic NPY/AgRP neurons, the ARC also contains specialized neurons which co-express pro-opiomelanocortin (POMC) and the peptide cocaine- and amphetamine-regulated transcript (CART). The POMC gene product is further processed into several short peptides, including the potently anorexigenic alpha-melanocyte-stimulating hormone (α-MSH), which specifically acts through the MC3R and MC4R melanocortin receptors. The central melanocortin pathway is the best-characterized neuronal pathway involved in the control of energy balance, and has been reviewed extensively [57].

Nerve terminals from ARC NPY neurons densely innervate neighbouring ARC POMC neurons [60] (Fig. 1). NPY directly activates Y1 and Y2 receptors expressed by POMC neurons, leading to membrane hyperpolarization and an inhibition of firing activity [2,60,107,244]. Additional involvement of the Y5 receptor has been implicated in the regulation of spontaneous release of α-MSH from POMC neurons [101], further supporting the NPY-mediated inhibition of the melanocortin system. In addition, AgRP itself acts as an endogenous antagonist of both MC3R and MC4R ([98], blocking the actions of α-MSH in downstream hypothalamic nuclei, and inhibiting POMC neurons directly through the MC3Rs that they express [139]. Similarly, it has been demonstrated that the melanocortin receptors MC3R and MC4R are present on ARC NPY neurons [215], suggesting reciprocal regulation of these oppositional systems in the ARC (Fig. 1). Thus, suppression of the primary anorexigenic system in the ARC is one mechanism by which NPY mediates its orexigenic activity in the hypothalamus, though it has been