



PAPER

The NPY/AgRP neuron and energy homeostasis

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Kennedy hypothesized nearly 50 y ago that negative feedback regulation of body fat stores involves hormones that circulate in proportion to adiposity and enter the brain, where they exert inhibitory effects on food intake and energy balance. Recent studies implicate leptin and insulin as 'adiposity signals' to the brain that promote negative energy balance in two ways: by inhibiting 'anabolic' hypothalamic neuronal circuits that stimulate food intake and promote weight gain, and by activating 'catabolic' pathways that reduce food intake and body weight. Chief among candidate 'anabolic' effector pathways is the NPY/AgRP neuron, found only in the hypothalamic arcuate nucleus. These neurons make peptides that potently stimulate food intake not only by increasing neuropeptide Y (NPY) signaling, but by reducing melanocortin signaling via the release of agouti-related peptide (AgRP), an endogenous melanocortin 3/4 receptor antagonist. Since NPY/AgRP neurons express receptors for leptin and insulin and are inhibited by these hormones, they are activated by a decrease of leptin or insulin signaling. Fasting, uncontrolled diabetes, and genetic leptin deficiency are examples of conditions in which food intake increases via a mechanism hypothesized to involve NPY/AgRP neurons. Data are reviewed which illustrate the role of these neurons in adaptive and maladaptive states characterized by hyperphagia and weight gain.

International Journal of Obesity (2001) 25, Suppl 5, S56–S62

Keywords: energy homeostasis; adiposity

Introduction

A large and compelling body of evidence supports the hypothesis that hormonal signals generated in proportion to body fat stores act in the central nervous system (CNS) via a negative feedback loop to regulate energy homeostasis. Initial evidence in support of this concept stemmed from studies of the adaptive response to conditions characterized by negative energy balance and weight loss. Included in these responses are an increase of food intake and reduction of energy expenditure that enable efficient recovery of lost weight comprising an important protective mechanism for survival. Hypothalamic neurons activated by weight loss play an especially important role in this response, and include a subset of neurons in the arcuate nucleus that synthesize both neuropeptide Y (NPY) and agouti-related peptide (AgRP). This paper reviews the regulation of the NPY/AgRP neuron in energy homeostasis and its contribution to compensatory responses induced by changes in energy balance and body fat stores.

Negative feedback control of body fat stores

The hypothesis that body adiposity is regulated by circulating factors released in proportion to adipose tissue mass that act in the brain to maintain energy balance was first proposed by Kennedy in 1953.¹ This model received subsequent support from 'parabiosis' experiments conducted by Coleman in the 1970s, from which he concluded that genetically obese *ob/ob* mice lack a circulating factor that inhibits feeding, whereas mice with obesity due to a different single gene mutation (*db/db*) are resistant to this factor.^{2,3} In 1994, Zhang and colleagues successfully cloned the *ob* gene, mutation of which causes obesity in the *ob/ob* mouse and encodes the adipose tissue hormone, leptin.⁴ Shortly thereafter, leptin receptors were shown to be concentrated in key hypothalamic areas and the *db* mutation was localized to the leptin receptor gene.⁵ Since leptin is secreted from adipocytes in proportion to body adiposity, and since both leptin deficiency and leptin receptor mutation cause severe genetic obesity in mice, these results support a key role for leptin as an adiposity signal to the brain.

The pancreatic hormone insulin is also implicated in the central nervous system (CNS) control of body adiposity. Like leptin, insulin is secreted in proportion to body adiposity^{6,7} and enters the CNS in proportion to its plasma level.⁸ In addition, leptin receptors and insulin receptors are expressed

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by neurons in brain areas known to be involved in feeding^{9,10} and central administration of either leptin^{11,12} or insulin¹³ reduces food intake and body weight. Further, neuron-specific loss of insulin receptors causes excess body fat stores,¹⁴ as does genetic deficiency of insulin receptor substrate-2 (IRS-2),¹⁵ a key molecule in the insulin receptor signaling pathway. Several observations support the hypothesis that NPY/AgRP neurons participate in the hypothalamic response to leptin and insulin action.

Central effector pathways for energy homeostasis

Within the CNS, a variety of neuropeptides have been shown to increase food intake while others have an anorectic effect (Table 1), and several of these can influence energy expenditure as well. ‘Anabolic’ pathways are defined here as those that promote positive energy balance by both stimulating food intake and reducing energy expenditure. Conversely, ‘catabolic’ pathways promote negative energy balance by inhibiting food intake and increasing energy expenditure. Importantly, the former pathways are proposed to be inhibited by input from insulin and leptin, while the latter are stimulated by these hormones. In response to conditions in which insulin and leptin signaling are reduced (eg fasting), anabolic pathways are therefore activated while catabolic pathways are inhibited, and it is via this set of integrated responses that depleted body fat stores are gradually replenished (Figure 1). Criteria that define candidate anabolic effector pathways include: (i) Localization to brain areas involved in energy homeostasis; (ii) Activation promotes positive energy balance through increases of food intake and decreases of energy expenditure; (iii) Inhibition by adiposity signals such as insulin and leptin and activation by energy restriction and/or deficiency of these signals; and (vi) Genetic or pharmacological impairment of the candidate

Table 1 Candidate neuropeptides and neurotransmitters implicated in the hypothalamic control of energy homeostasis

Orexigenic/anabolic	Anorectic/catabolic
NPY ^{a,b}	α -MSH ^{c,d}
AgRP ^{a,b}	CRH ^{c,d}
MCH ^a	TRH ^{c,d}
Orexin A and B ^a	CART ^{c,d}
Galanin ^{a,b}	GLP-1 ^c
Noradrenalin ^a	Neurotensin ^c
Ghrelin ^a	Serotonin ^a
Beacon ^a	Oxytocin ^c

^aIncreased food intake; ^bdecreased energy expenditure; ^cdecreased food intake; ^ddecreased energy expenditure.

NPY, neuropeptide Y; AgRP, agouti-related peptide; MCH, melanin concentrating hormone; α -MSH, alpha-melanocyte stimulating hormone; CRH, corticotropin-releasing hormone; TRH, thyroid-releasing hormone; CART, cocaine- and amphetamine-related transcript; GLP-1, glucagon like peptide-1.

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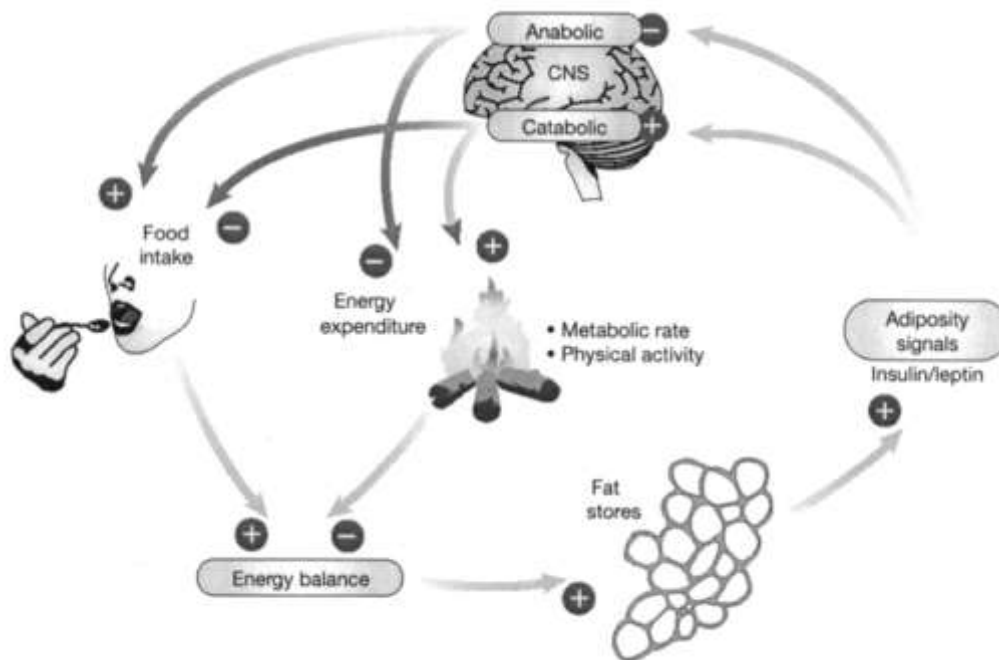


Figure 1 Model for the regulation of energy balance and adiposity. The adiposity signals insulin and leptin are secreted in proportion to body fat content and act in the hypothalamus to inhibit anabolic and stimulate catabolic, effector pathways. Modified from Schwartz *et al.*⁷⁵

pathway reduces food intake and/or attenuates responses to a deficiency of adiposity signals. Hypothalamic NPY-containing neurons were the first to meet these criteria.

Neuropeptide Y

Neuropeptide Y (NPY) is a 36 amino acid peptide isolated originally from porcine brain¹⁶ that is synthesized in both peripheral and central neurons. The major sites for the stimulatory effects of NPY on food intake are within the hypothalamus. Hypothalamic NPY is synthesized primarily in arcuate nucleus neurons, although NPY-containing projections from the brainstem are also described.¹⁷ Arcuate nucleus NPY neurons project to adjacent hypothalamic areas such as the paraventricular nucleus (PVN), dorsomedial nucleus (DMN), perifornical area (PFA), lateral hypothalamic area (LHA) and the medial preoptic area (MPO),^{17,18} major integration areas for the regulation of both feeding behavior and energy expenditure.

NPY is one of the most potent orexigenic agents known. A single bolus intracerebroventricular (i.c.v.) injection of NPY potently stimulates feeding in rodents¹⁹ and other mammalian species including sheep and monkeys.^{20,21} Repeated injection of NPY directly into the PVN over a period of several days results in sustained hyperphagia, body weight gain and a marked increase of body fat accumulation.²² Some of NPY's anabolic effects also arise from decreased heat production in brown adipose tissue (BAT)^{23,24} via inhibition of sympathetic nervous system (SNS) outflow to this thermogenic tissue. Thus, NPY promotes net energy gain by simultaneously increasing energy intake and decreasing energy expenditure.

Chronic central NPY administration also exerts effects in peripheral tissues that promote fat deposition. These include increased acetyl coenzyme-A carboxylase activity and *de novo* fatty acid and triglyceride synthesis in both white adipose tissue and the liver. Some of these effects of central NPY administration may arise in part from the increased secretion of glucocorticoids from the adrenal gland, which exacerbates the insulin resistance, hypertriglyceridemia and hyperinsulinemia²⁵⁻²⁷ induced by chronic central NPY administration. NPY is also reported to elevate circulating insulin concentrations, and thereby to favor further the deposition of triglycerides in white adipose tissue.^{28,29} This effect is associated with increased insulin-stimulated glucose uptake by adipose tissue but decreased glucose uptake by muscle *in vivo*.^{25,26}

Increased food intake cannot fully account for the impressive metabolic and hormonal responses to NPY administration. This conclusion is supported by studies in which NPY-induced hyperphagia was prevented by pair-feeding animals to the food intake of vehicle-treated controls, yet the metabolic and hormonal responses induced by NPY were only partially ameliorated.³⁰ The mechanisms responsible for these metabolic effects are not fully known but are likely to involve changes of autonomic activity and glucocorticoid release.

By comparison, interventions that reduce NPY signaling in the CNS, such as i.c.v. administration of antisense oligonucleotides complementary to NPY mRNA, lower cumulative food intake, meal size, meal duration and body weight gain in rats.³¹ Similar responses were reported following i.c.v. infusion of NPY antisera,³² and these data collectively suggest a physiological role for NPY to increase food intake. The hyperphagic response to NPY involves signaling at multiple NPY receptor subtypes. Of six known NPY receptors (from Y1 to Y6), the Y1 and Y5 isoforms are most strongly implicated in NPY's feeding effects. NPY Y1 receptor antagonists potently suppress NPY-induced feeding³³⁻³⁵ and mice that lack Y1 receptors due to targeted gene knockout exhibit both a suppression of NPY-induced feeding and impaired refeeding following a fast.^{36,37} Thus, NPY probably exerts at least some of its feeding effects via the Y1 receptor. Surprisingly, however, Y1-deficient animals also develop a mild form of obesity, and neither the mechanism underlying this outcome nor its relevance to the neurobiology of NPY action is understood. NPY Y5 receptor antagonists also inhibit food intake, but surprisingly have no effect on NPY-induced feeding responses,³⁸ raising questions about the role of Y5 in NPY's feeding effects. However, NPY Y5 receptor selective agonists stimulate feeding^{39,40} and NPY Y5-deficient mice do have blunted feeding in response to NPY.⁴¹ Taken together, these data suggest a role for both Y1 and Y5 receptors in the orexigenic actions of NPY.

Hypothalamic biosynthesis and release of NPY is regulated by a variety of stimuli in a manner consistent with its proposed role in energy homeostasis. NPY gene expression is upregulated during periods of negative energy balance such as fasting,⁴² insulin dependent diabetes induced in rats by the β -cell toxin, streptozotocin (STZ)⁴³ and lactation.⁴⁴ A role for NPY in the hyperphagic response exhibited during these conditions can therefore be considered.

A key role for leptin as an inhibitor of hypothalamic NPY biosynthesis is supported by studies of rodent models of genetic obesity due to defective leptin signaling. NPY mRNA concentrations are increased in the arcuate nucleus of mice with obesity due to genetic leptin deficiency (*ob/ob*),^{45,46} or leptin receptor mutation in mice (*db/db*)⁴⁷ and rats (*fa/fa*).⁴⁸ Furthermore, leptin treatment at doses that reduce food intake, body weight and body fat content, also inhibit arcuate nucleus NPY gene expression.^{46,49} This leptin-induced reduction of NPY gene expression is not mediated by reduced food intake and body weight, since pair-feeding of vehicle-treated *ob/ob* mice to the intake of leptin-treated animals produced equivalent weight loss but did not alter NPY mRNA expression.⁴⁶ Leptin treatment was ineffective in reducing either food intake or NPY mRNA levels in leptin-resistant *db/db* mice, demonstrating that functional leptin receptors are required for these responses. Moreover, when mice lacking NPY are crossed onto the leptin-deficient *ob/ob* background, the hyperphagia and obesity characteristic of *ob/ob* mice is sharply attenuated. Thus, the full response to leptin deficiency appears to require NPY signaling.⁵⁰

The hypothesis that leptin acts directly upon NPY neurons to inhibit NPY gene expression is supported by the co-expression of leptin receptor mRNA with NPY mRNA in the arcuate nucleus.⁵¹ Approximately 50% of the arcuate nucleus NPY neurons in rats express the 'long' or 'signaling' leptin receptor isoform (Ob-Rb) and it is this subpopulation of arcuate nucleus NPY neurons that is activated by fasting.¹⁰ By comparison, NPY neurons outside the hypothalamus do not appear to express leptin receptors and are not activated by fasting.

Insulin is also implicated as a physiological inhibitor of hypothalamic NPY neurons. Like leptin, central insulin administration inhibits arcuate nucleus NPY gene expression in both food-deprived⁵² and STZ diabetic rats, a model of diabetic hyperphagia.⁵³ These findings collectively support the hypothesis that NPY plays a key role in stimulating food intake when energy stores are threatened, as signaled by a decrease of inhibitory input from adiposity signals, such as leptin and insulin. Moreover, insulin and leptin appear to subserve overlapping functions as inhibitors of arcuate nucleus NPY neurons.

A key role for NPY in the normal control of food intake is challenged, however, by evidence that mice lacking NPY have normal daily food intake and body weight and exhibit normal 24 h hyperphagia and body weight recovery after a 48 h fast.⁵⁴ Leptin's ability to reduce food intake, body weight and body fat is not compromised in NPY-deficient mice. On the other hand, leptin deficiency expresses its full phenotype only in NPY competent animals, as discussed above. These findings suggest that NPY is not required for leptin's inhibitory effects in many circumstances and that other factors may play an important role in the response to leptin and in regulation of body weight.

Recent data reveal that, although NPY knockout mice exhibit normal daily food intake, they display abnormal feeding behavior in response to stimuli that are independent of a decrease of body adiposity. The effect of uncontrolled diabetes to increase food intake (diabetic hyperphagia) is attenuated in NPY knockout mice treated with STZ when compared with diabetic wild-type mice.⁵⁵ These findings collectively suggest a key role for NPY in the ability of some stimuli to increase food intake, but in response to fasting, NPY-deficient mice compensate by recruiting redundant signaling molecules. One such compensatory effector is the melanocortin receptor (mcr) antagonist, AgRP.

Agouti-related peptide and the melanocortin system

The cloning of the AgRP gene in 1997 identified a 132 amino acid peptide with 25% homology to the agouti protein (also known as agouti-signaling protein), but unlike agouti, AgRP is normally found in the hypothalamus.^{56,57} Agouti is a protein that normally is expressed exclusively in hair follicles and acts in a paracrine fashion to inhibit alpha-melanocyte stimulating hormone (α -MSH)-induced eumelanin (black)

deposition and thereby favors pheomelanin (yellow) production and yellow hair color.^{58,59} This effect is accomplished via competitive antagonism of the melanocortin-1 receptor (Mc1r). AgRP exerts its effects on energy homeostasis via an analogous effect on the brain melanocortin signaling system. By antagonism of the melanocortin-3 receptor (Mc3r) and melanocortin-4 receptor (mc4r), AgRP stimulates food intake.

Melanocortins comprise a family of peptides that include α -MSH and adrenocorticotrophic hormone (ACTH), which are cleaved from the polypeptide precursor, pro-opiomelanocortin (POMC). In the brain, POMC expression occurs only in the nucleus of the solitary tract (NTS) in the brainstem and in the arcuate nucleus. Since arcuate nucleus POMC mRNA expression is reduced in *ob/ob* and *db/db* mice and in conditions associated with reduced leptin signaling such as fasting and energy restriction,^{60,61} the melanocortin system appears to be stimulated by leptin. The finding that arcuate nucleus POMC gene expression is increased by leptin^{60,62} and that POMC mRNA is co-localized with leptin receptor mRNA in arcuate nucleus neurons,⁶³ provides direct support for this hypothesis.

Melanocortins were first implicated in energy homeostasis after Mc3r and Mc4r were identified and found to be expressed primarily in the brain.^{64,65} Further evidence included the findings that melanocortin receptor agonists reduce food intake⁶⁶ while central administration of melanocortin receptor antagonists stimulate feeding.^{66,67} Since Mc4r-deficient mice develop a hyperphagic obesity syndrome,⁶⁸ melanocortin signaling in the brain appears to be required for normal control of food intake and body weight.

In the brain, AgRP is synthesised exclusively in the arcuate nucleus⁵⁶ by neurons that project to adjacent hypothalamic areas such as the PVN, DMN, and LHA. Thus, neurons synthesizing AgRP, POMC and NPY are localized to a small area of the brain that appears to play a key role in the CNS response to insulin and leptin. The demonstration that AgRP mRNA is abundantly co-localized with NPY, but not POMC mRNA^{69,70} identifies NPY/AgRP neurons as a unique subset that is capable of increasing food intake via two different mechanisms: by increasing NPY signaling on the one hand, and decreasing melanocortin signaling on the other.

Like NPY, AgRP is overexpressed in the arcuate nucleus of mice with either leptin deficiency (*ob/ob*) or leptin receptor mutation (*db/db*) and is also upregulated by fasting in normal mice and rats.^{56,57,71} AgRP mRNA expression is decreased following leptin treatment of *ob/ob*, but not *db/db*, mice as described for NPY.⁶¹ Thus, the co-expressed orexigenic molecules NPY and AgRP have redundant effects on feeding behavior, exhibit similar patterns of regulation by leptin and are likely to be major targets of leptin action in the brain. However, increases of AgRP mRNA in response to both fasting and genetic leptin deficiency are substantially greater than observed for NPY mRNA levels, whereas such differences are not apparent in STZ-diabetic rats.⁷² Thus, although NPY and AgRP are both upregulated in the arcuate

nucleus in response to the same stimuli, the magnitude of this response may differ. Distinct mechanisms may therefore govern the control of NPY and AgRP biosynthesis.

Differences in the magnitude and time-course of feeding in response to AgRP and NPY are also apparent. Specifically, while NPY is more potent in the short-term, central administration of AgRP induces hyperphagia for a duration that is unprecedented in comparison to other known orexigenic stimuli. The increase of food intake following a single i.c.v. injection of AgRP is sustained for up to a week,^{73,74} while the response to NPY is sustained over hours, rather than days. Hagan and colleagues showed that the onset of hyperphagia induced by AgRP is blocked by co-administration of a Mc4r agonist (MT-II), but if MT-II is given 24 h after AgRP administration, the AgRP-induced increase of food intake is not affected.⁷⁴ This observation suggests that AgRP's sustained feeding response may involve mechanisms that are ultimately independent of Mc4r signaling.

Conclusion

Arcuate nucleus NPY/AgRP neurons appear to play an important role in energy homeostasis, transducing changes of body fat content (as communicated by altered insulin and leptin levels) into compensatory feeding responses.⁷⁵ Several features distinguish these cells from other NPY neurons in the brain. In addition to co-expression of AgRP, these are the only NPY neurons known to express the long form of the leptin receptor and to be activated by negative energy balance. It is in this setting that the NPY/AgRP neuron is proposed have its most important role, as these neurons can increase food intake and body weight by simultaneously stimulating NPY signaling and inhibiting melanocortin signaling in the hypothalamus. Consequently, disturbances of energy homeostasis due to defective inhibition of the arcuate nucleus NPY/AgRP neuron can have major consequences, including hyperphagia, reduced energy expenditure, disturbances of glucose and lipid metabolism and obesity. The growing prevalence of obesity in human populations highlights the potential importance of the NPY/AgRP neuron in normal and abnormal energy homeostasis.

Acknowledgements

This work was supported by NIH grants DK 52989, NS 32273 and DK 12829.

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