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Low Abundance of NPY in the Hypothalamus can Produce Hyperphagia and Obesity

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Abstract

States of increased metabolic demand are associated with up-regulation of NPY and hyperphagia. However, we present some instances of hyperphagia in which NPY is not up-regulated. Ablation or functional disruption of specific sites in the hypothalamus, such as the ventromedial or paraventricular nuclei, or transection of inputs to the hypothalamus from the hindbrain results in hyperphagia and excess body weight gain. However, NPY expression and concentration in these experimental models is either decreased or unchanged. While there is no up-regulation of NPY in these models, there is increased sensitivity to the orexigenic effects of NPY. This enhanced responsiveness to NPY may more than compensate for the reduced levels of NPY and result in hyperphagia and excess body weight gain. The hyper-responsiveness may be due either to an increase in NPY receptors or to other changes in target cells and response pathways that may result from the treatments used in these models.

Keywords

food intake; NPY Y1 receptor; neural transection; melanocortin system

1. Introduction

Up-regulation of NPY in the hypothalamus is well established in many instances of hyperphagia and increased metabolic demand such as fasting, diabetes, lactation and exercise [22,24,26]. The paraventricular nucleus of the hypothalamus (PVN) has been shown to be a major site of action of the interacting hypothalamic neurochemical circuits regulating food intake [3,26,42]. Fasting elicits a gradual, time-related increase in NPY levels in the PVN; one day of refeeding returns NPY levels to the range found in control satiated rats [33]. Likewise, in food-deprived rats both the in vitro release of NPY from the microdissected PVN and the in vivo NPY release from the PVN are increased and refeeding returns release to control levels [8,25]. These effects of fasting are accompanied by increased hypothalamic NPY mRNA expression [39]. Rats made diabetic by streptozotocin injection are also hyperphagic [36,40]. We have found that both NPY concentration and in vivo release in the PVN of streptozotocin-induced diabetic rats are increased [36]. Further, NPY mRNA expression in the arcuate nucleus, a site of NPY neurons projecting to the PVN, is also increased in these rats [40].

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Hypothalamic NPY systems are up-regulated during increased energy demand as in lactating rats that exhibit profound hyperphagia [4,5]. Pharmacological studies using a peptide analogue of NPY that antagonizes NPY-induced feeding have provided evidence for a role of NPY in the hyperphagia of lactation [4]. Similarly, increased metabolic demand due to intense physical exercise is accompanied by increased hypothalamic NPY concentrations [28]. In addition to the conditions mentioned above, obesity due to genetic factors is often associated with hyperphagia and increased NPY signaling in rodents [17,26].

Thus, it is well established that an increased abundance of NPY plays a role in increasing energy intake in instances of increased metabolic demand [16,26]. However, high abundance of NPY is not invariably required for the occurrence of hyperphagia or obesity. Below we present some models in which a low abundance of NPY is associated with hyperphagia and excess body weight gain.

2. Models of hyperphagia with low abundance of NPY

2.1. Neural transection at the level of mesencephalon

Increasing evidence suggests that bi-directional communication between the hypothalamus and hindbrain participates in the daily regulation of food intake and energy balance [2,13,14, 41,43]. Interrupting neural connections between the hindbrain and the hypothalamus by bilateral neural transection at the level of the dorsal tegmentum in the mesencephalon produces hyperphagia and excess body weight gain [32,38]. These effects develop gradually with the increase in body weight typically requiring two to three weeks after the transection surgery to be fully manifested [32, unpublished observations]. Interestingly, the hyperphagia only occurs during the dark phase, but nonetheless results in an approximately 45% increase in daily food intake [32]. Consistent with the increased body weight, both serum leptin and insulin are increased, but there is no change in the daily pattern of their secretion [32].

These transections interrupt NPYergic input from the hindbrain to the hypothalamus [35]. Using the micropunch technique, we have found that NPY concentrations two weeks following transection are markedly decreased (50–60%) in the PVN, medial preoptic area, median eminence and dorsomedial nucleus [35]. This reduction in NPY concentration is especially noteworthy since it includes the PVN, which is a major site of NPY action in the regulation of food intake [23,26].

We have found that rats with these bilateral neural transections are hyper-responsive to the orexigenic effects of NPY. Injection of NPY into the third cerebroventricle of transected rats produces a greater than 50% decrease in the latency to onset of feeding and an almost two fold increase in the amount of food consumed compared to sham controls [34]. Thus, while the amount of NPY in feeding related hypothalamic sites, such as the PVN, may be decreased, the increased responsiveness to NPY may be sufficient to more than compensate for the decreased input and thus lead to the hyperphagia transected rats display.

2.2. Electrolytic lesion of the ventromedial hypothalamus (VMH)

The classic ‘hypothalamic obesity’ or ‘VMH obesity’ syndrome follows electrolytic lesioning of the ventromedial area of the hypothalamus. Animals receiving this treatment become extremely obese and exhibit hyperphagia immediately following the lesioning surgery [31]. Rats with these lesions rapidly become hyperinsulinemic and hyperleptinemic in parallel with the rapid rise in body weight [12].

NPY mRNA levels in the medial basal hypothalamus of VMH-lesioned rats are markedly decreased relative to controls, however, the daily rhythm of NPY mRNA expression is maintained. NPY concentrations of VMH-lesioned rats are significantly decreased in several

feeding relevant hypothalamic nuclei. In the dorsomedial nucleus and the lateral hypothalamic area NPY concentrations are significantly decreased by day 7 post-lesion and these decreases continue through day 21 post-lesion [12]. The median eminence-arcuate nuclei of lesioned rats also have significantly decreased NPY concentrations 7 days following the lesioning surgery. In the PVN, NPY concentrations are significantly decreased relative to controls within 2 days post-lesion and remain depleted through day 21 and NPY release in vitro from the microdissected PVN is significantly reduced [12].

However, despite these reduced levels, NPY plays a significant role in the hyperphagia of VMH-lesioned rats. Blockade of NPY action by passive immunization against NPY via intracerebroventricular administration of NPY antibodies controls the hyperphagia and restores food intake to control levels [9].

2.3. Colchicine microinjection into the VMH

Disruption of axoplasmic flow in the VMH with the neurotoxin colchicine [29,30] produces a transient hyperphagia lasting approximately four days with a corresponding increase in body weight [1,20]. As in the case of electrolytic lesion of the VMH, serum insulin and leptin levels rapidly increase in colchicine-injected rats during the period of hyperphagia and increasing body weight [6,21].

NPY mRNA expression in the median eminence-arcuate region of colchicine-injected rats is significantly decreased from day 2 post-injection onward and NPY concentration in the microdissected PVN is decreased [20]. Using the push-pull perfusion technique, we have found that in vivo NPY release from the PVN is significantly suppressed in these rats [19]. Thus, colchicine injection into the VMH results in rapid down regulation of NPY in the hypothalamus.

As in the case of rats with neural transactions disrupting input to the hypothalamus, colchicine-injected rats are hyper-responsive to the orexigenic effects of NPY. Intracerebroventricular injection of NPY produced a several fold increase in food intake in colchicine-injected rats relative to saline-injected rats at three different doses of NPY [20]. An injection of 29 pmole of NPY, a dose which is ineffective in control rats, produced substantial food intake in colchicine-injected rats [20]. Thus, while NPY is down regulated in colchicine-injected rats, the increased sensitivity to the orexigenic effects of NPY may be more than enough to compensate for the decreased NPY and contribute to the hyperphagia observed. Evidently an increase in NPY Y1 receptors contributes to this increased sensitivity to NPY. Analysis of NPY receptor mRNA expression in the medial basal hypothalamus revealed a significant increase in Y1 receptor mRNA in colchicine-injected rats [21]. Blockade of Y1 receptors with a Y1 receptor antagonist significantly reduced food intake in these rats [20].

Changes in other feeding-related hypothalamic neuropeptides may also contribute to the hyperphagia. The PVN is a major site of action and interaction of the NPY and melanocortin systems [3,26,42]. The melanocortin system, acting through the POMC gene product α-MSH, is believed to be a major anorexigenic component of the hypothalamic appetite regulating circuitry [3,26,42]. Electrophysiological studies demonstrate that individual neurons in the PVN respond to and integrate melanocortin and NPY signals [3]. The Y1/Y5 receptors for NPY and MC3/MC4 receptors for α-MSH in the PVN are targets of NPY and POMC neurons in the arcuate nucleus [3,18,24,26,42]. POMC mRNA in the arcuate nucleus is significantly decreased in colchicine-injected rats [10]. Levels of α-MSH are decreased in the micropunched PVN, dorsomedial nucleus, and perifornical hypothalamus, sites implicated in the control of food intake [10]. Intracerebroventricular injection of the MC3/MC4 melanocortin receptor agonist MTII prevented the hyperphagia and body weight gain seen in colchicine-injected rats [10]. Thus, the hyperphagia observed in colchicine-injected rats may be due to a combination

of increased sensitivity to NPY and decreased melanocortin restraint on the orexigenic action of NPY.

2.4. Electrolytic lesion of the PVN

Bilateral electrolytic lesions of the PVN result in hyperphagia and increased body weight gain [7,27]. Profound hyperphagia occurs immediately following the lesioning surgery and continues for about a week after which it decreases and stabilizes at a lower level for several more weeks [7,11]. Body weight changes follow a parallel time course [7,11]. However, unlike the VMH-lesioned rats, hypothalamic NPY mRNA expression and NPY concentrations are unchanged in PVN-lesioned rats relative to sham-operated controls [11].

We assessed the orexigenic action of exogenous NPY in these hyperphagic PVN-lesioned rats. As in the cases discussed above, there was an increased feeding response following cerebroventricular injection of NPY in lesioned rats relative to the sham-operated controls [7]. The feeding response of the PVN-lesioned rats was nearly three fold greater relative to the controls at doses of 118 and 470 pmole of NPY (Fig. 1) [7]. Thus, as in the cases discussed above, while there is no up-regulation of NPY in PVN-lesioned rats, increased sensitivity to the orexigenic effects of NPY may contribute to the hyperphagia observed in this model.

Although the PVN is believed to be a major site of action and interaction of the NPY and melanocortin systems [3,26,42], despite the absence of the PVN in the lesioned rats, sensitivity to the orexigenic effects of NPY is increased and, furthermore, melanocortin suppression of food intake was not altered [7] suggesting other target site(s) for the actions of these ligands. NPY has been shown to stimulate food intake when injected into many areas of the hypothalamus [37]. The dorsal vagal complex in the brainstem contains a high concentration of melanocortin receptors and is a site of POMC mRNA expression [13,41,43]. Thus, a broader view of possible sites of action of NPY and other feeding regulating neuropeptides may be necessary as the PVN does not appear to be essential for the orexigenic and anorexigenic effects of NPY and the melanocortin system.

3. Conclusions

The above models represent decreases in NPY levels produced by significant damage to various areas of the brain. These effects are not simply the result of extensive damage to neural tissue, but can occur with an intact normally functioning brain. Significant decreases in NPY concentration can also be produced by feeding a high-fat diet [15]. Rats maintained on a high-fat diet for nine weeks or longer have significantly decreased hypothalamic NPY content while showing an increased feeding response to NPY injected into the cerebroventricles [15].

While the reduction in hypothalamic NPY levels in the rats with bilateral neural transections may result from reduced NPY input from the hindbrain, the cause of the reduced NPY levels in the electrolytically-lesioned and colchicine-injected VMH models is not completely clear. Major clusters of NPY-producing neurons are not affected by these treatments, although it is possible that NPYergic fibers passing through these sites are affected. Alternatively, the elevation of negative feedback signals such as leptin created by the hyperphagia and excess body weight may act to produce the decrease in NPY levels. Likewise, the cause of the increased feeding response to exogenous NPY has not been elucidated. It is likely that increased NPY Y1 receptor up-regulation, as noted in VMH colchicine-injected rats, may contribute to the hyper-responsiveness in the other models. It is also possible that these treatments produce other changes in the responsive cells that produce increased food intake in response to NPY administration. For example, removal of anorexigenic input, such as may result from the decreases in α-MSH levels as seen in colchicine-injected rats, may contribute to the increased feeding response to NPY.

To summarize, ablation or functional disruption of specific sites in the hypothalamus or transection of inputs to the hypothalamus results in hyperphagia and excess weight gain. However, NPY expression and concentration in these experimental models is either decreased or unchanged. While there is no increase in NPY in these models, we have found disruption of input from the hindbrain to the hypothalamus, disruption of signaling in the VMH and ablation of the PVN all result in increased sensitivity to the orexigenic effects of NPY. This enhanced responsiveness to NPY may more than compensate for the reduced levels of NPY and result in hyperphagia and excess body weight gain. The hyper-responsiveness may be due either to an increase in NPY receptors or to other changes in target cells and response pathways that may result from the treatments used in these models.

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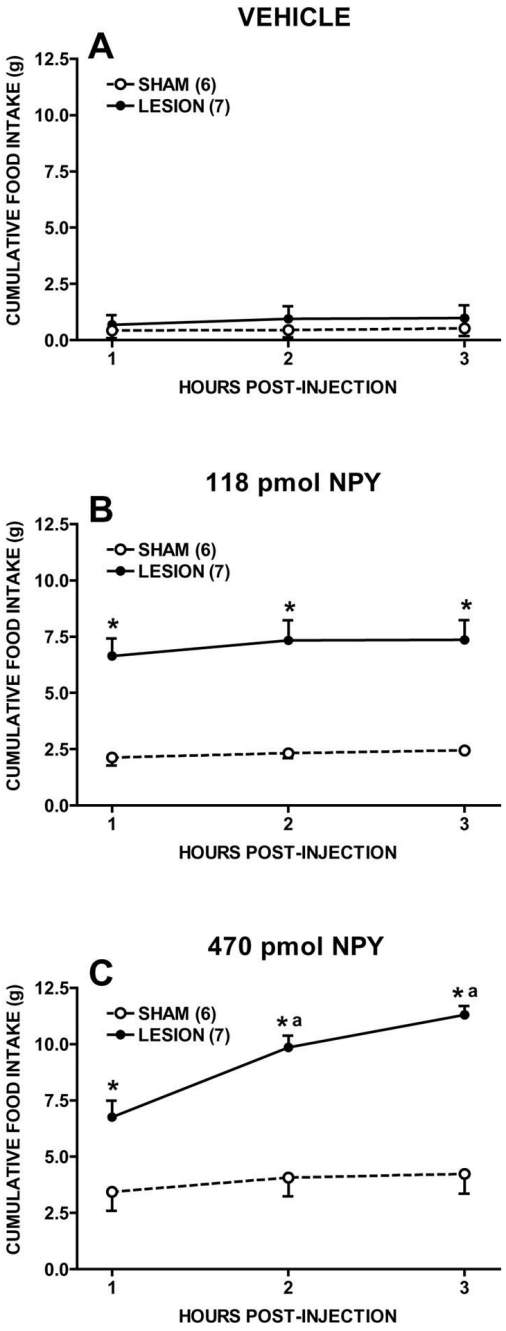

Fig 1.

Enhanced food intake in PVN-lesioned as compared to sham-lesioned rats in response to intracerebroventricular injection of different doses of NPY. * p 0.05 vs. shams. [a] $p<0.05$ vs. food intake at 1 h post-injection. Reprinted from reference #7 with permission from Elsevier.