Brief Cutting Edge Report
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

Limbic Substrates of the Effects of Neuropeptide Y on Intake of and Motivation for Palatable Food

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Objective: Neuropeptide Y (NPY), given centrally augments food intake and the motivation to work for palatable food. Here, the brain regions were identified through which NPY increases food intake and motivation.

Methods: NPY was infused into three brain regions implicated in food intake and motivation: the lateral hypothalamus (LH), nucleus accumbens shell (NAc), and ventral tegmental area (VTA). Motivation for sucrose was assessed using a progressive-ratio schedule of reinforcement in which the effort to obtain successive rewards increased incrementally. To disentangle the effects of NPY on motivation for palatable food from food consumption, free-feeding experiments were performed in which animals had *ad libitum* access to sucrose pellets.

Results: Infusion of NPY into either VTA or NAc increased the motivation to respond for sucrose, whereas infusion of NPY in either NAc or LH increased sucrose consumption. In addition, the effect of intra-VTA NPY on motivation for food was attenuated after pretreatment with the dopamine receptor antagonist alpha-flupenthixol.

Conclusions: Specific limbic substrates through which NPY influences consumption of and motivation for palatable food were identified by these data. The motivational effects of NPY are exerted through the VTA, its consummatory effects through the LH, and the NAc is involved in both.

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Introduction

Predominantly expressed in the arcuate nucleus of the hypothalamus, Neuropeptide Y (NPY) plays a prominent role in the regulation of food intake (1). Through projections to various hypothalamic and extrahypothalamic regions, arcuate nucleus NPY-expressing neurons modulate food intake (2). In addition to its role in hypothalamic homeostatic regulation of food intake, a few studies have also shown a role for NPY in food-motivated behavior (3,4). Several hypothalamic regions are known to be involved in NPY-mediated food intake, but there is only scarce information on the brain areas that mediate the effects of NPY on food motivation (3,5). Here, we identified the neural substrates of NPY-driven motivation for food by infusing NPY into three brain regions implicated in food intake, reward, or motivation, that is the lateral hypothalamus (LH), nucleus accumbens shell (NAc), and ventral tegmental area (VTA), all of which contain NPY immunoreactivity and receptors (6).

The effect of NPY on the motivation for sucrose pellets was determined using a progressive-ratio schedule of reinforcement (7). To dissociate food motivation from consumption, the effect of intra-LH, -NAc, and -VTA NPY infusion was assessed in a sucrose-free-feeding experiment. As NPY receptors are expressed on dopaminergic neurons within the VTA (8) and that central infusion of NPY increases extracellular striatal dopamine levels (9), we further investigated whether the effects of intra-VTA NPY infusion were dopamine dependent. Here, animals were pretreated with saline or a dopamine receptor antagonist prior to infusions of NPY in the VTA.

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Additional Supporting Information may be found in the online version of this article.

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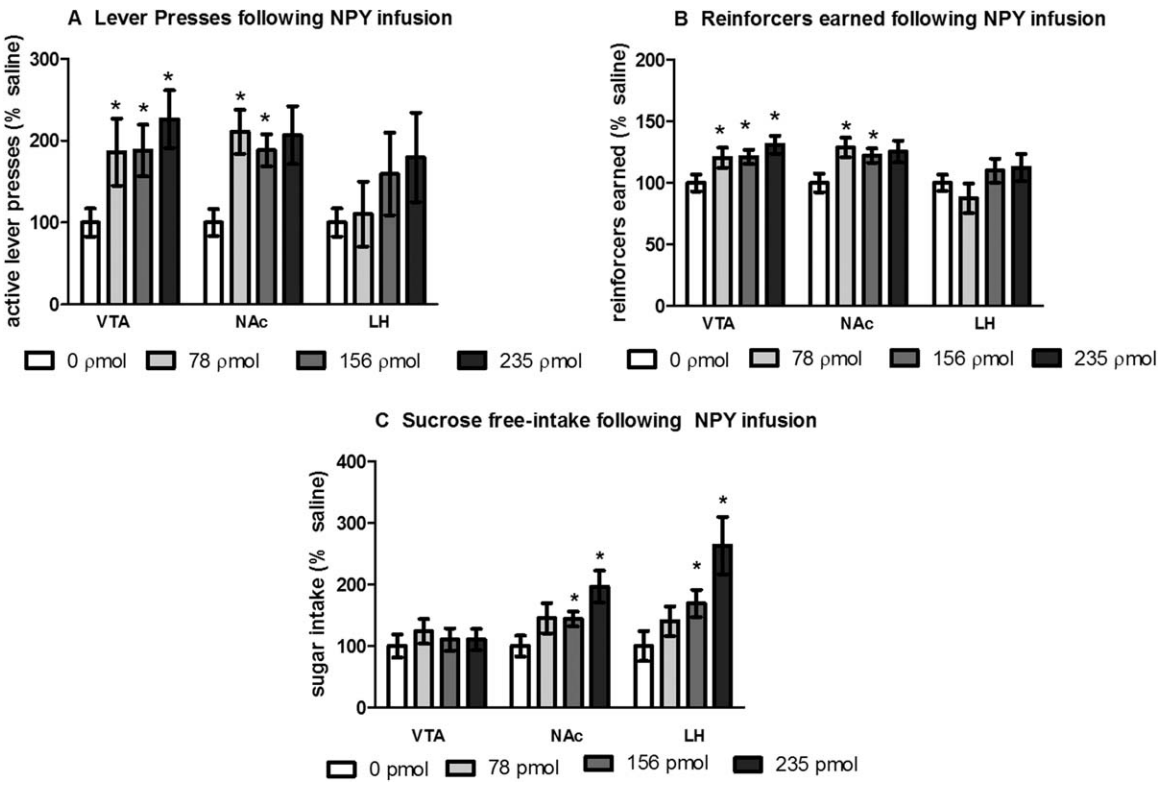

Figure 1 (**A**) active lever presses and (**B**) sucrose pellets earned upon infusion of NPY into the VTA (n = 11), NAc (n = 14), and LH (n = 10) expressed as percentage of saline infusion. (**C**) Free feeding of sucrose pellets in a 30-min period after saline versus infusion of NPY (expressed in percentage of saline infusion). *$P < 0.05$ compared to saline injections. Values are expressed as mean ± SEM.

Methods

For details of surgical procedures, microinfusion of drugs, and experimental designs, we refer to the Supporting Information data.

Animals

Male Wistar rats (Charles River, Germany) were maintained on *ad libitum* chow (3.31 kcal/g, SDS, United Kingdom) and housed individually (temperature, 21 ± 2°C) in light-controlled rooms (lights-off, 0700-1900 h). All experiments were approved by the Animal Ethics Committee, Utrecht University.

Drugs

Rat NPY (H-6375, Bachem, Germany) was dissolved in sterile saline and infused bilaterally in a counterbalanced fashion at doses of 0, 78, 156, and 235 pmol/300 ηl/side. Each animal received all doses of the drug. The doses were based on a previous study in which the effect of infusion of NPY into the perifornical hypothalamus on motivation for food was studied (3).

Statistics

All data were analyzed using SPSS (IBM, USA). Progressive-ratio data were analyzed using nonparametric Friedman's repeated measure analysis followed by Wilcoxon signed rank test corrected for multiple testing. Free-feeding data were analyzed using a repeated measures ANOVA followed by *post hoc* Bonferroni tests. A P-value of <0.05 was considered statistically significant.

Histology

After sacrifice, cannula placements were histologically verified using cresyl violet staining. Few animals (LH: 2 and NAc: 1) were excluded from the study owing to incorrect cannula placement.

Results

Effect of NPY on motivation for sucrose

Infusion of all doses of NPY into the VTA significantly increased the number of ALP ($\chi^2(3) = 18.25$ $P < 0.001$, Figure 1A) and reinforcers earned ($\chi^2(3) = 13.44$, $P < 0.05$, Figure 1B). Comparable to the VTA, infusion of NPY into the NAc significantly increased active lever presses ($\chi^2(3) = 10.46$, $P < 0.05$, Figure 1A) and reinforcers earned ($\chi^2(3) = 10.46$, $P < 0.05$, Figure 1B) at doses of 78 and 156 pmol. Infusion of NPY into the LH did not affect ALP ($\chi^2(3) = 4.44$, $P > 0.05$, Figure 1A) or the rewards earned ($\chi^2(3) = 6.00$ $P > 0.05$, Figure 1B). Inactive lever presses were not affected by infusion of NPY into any area (data not shown).

Effect of NPY on free feeding of sucrose

The amount of sucrose consumed was significantly higher after the infusion of NPY into the LH at the doses of 156 and 235 pmol

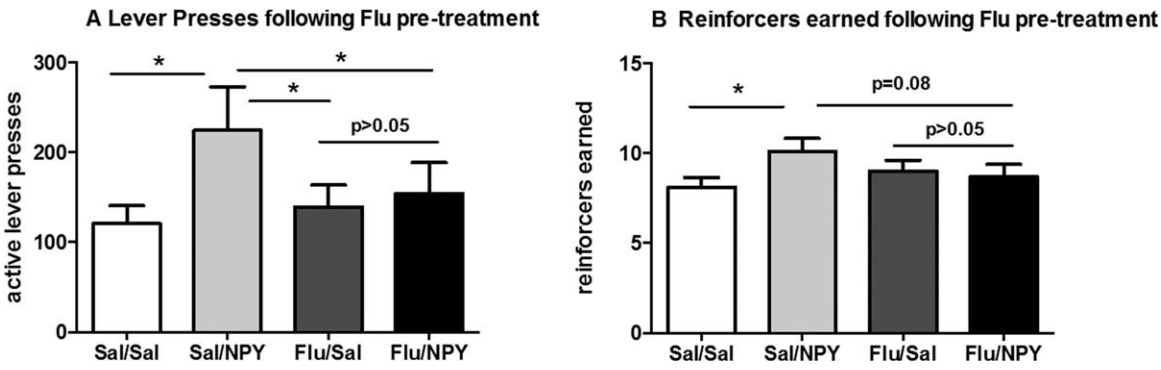

Figure 2 (**A**) Active lever presses and (**B**) rewards earned after treatment with alpha-flupenthixol (0.125 mg/kg, intraperitoneal) prior to infusion of NPY (156 pmol/side) into the VTA ($n = 10$).Asterisks indicate statistically significant ($P < 0.05$) differences between the various treatment conditions. Values are expressed as mean ± SEM.

($F_{(3, 21)} = 12.29$, $P < 0.05$, Figure 1C). Similar to the LH, infusion of NPY into the NAc enhanced the free intake of sucrose at the doses of 156 and 235 pmol ($F_{(3, 18)} = 6.27$, $P < 0.05$, Figure 1C). In contrast, infusion of NPY into the VTA did not alter free feeding ($F_{(3, 18)} = 0.36$, $P > 0.05$, Figure 1C).The 24-h chow intake after the infusion of NPY into all three brain areas remained unchanged (Supporting Information Figure S3).

The motivational effect of intra-VTA NPY is dopamine dependent

Infusion of NPY increased the number of ALP and rewards earned (Figure 2). Although alpha-flupenthixol pretreatment did not have an effect by itself, it attenuated the NPY-driven increase in ALP ($\chi^2(3) = 12.60$, $P < 0.05$, Figure 2A) and rewards earned ($\chi^2(3) = 14.68$, $P < 0.05$, Figure 2B).

Discussion

Here, we demonstrate a site-specific dissociation between the effects of NPY on sucrose consumption and the motivation for sucrose after administration into different limbic regions. Infusion of NPY into either VTA or NAc increased motivation to respond for sucrose, whereas it failed to do so after infusion into LH. In contrast, infusion of NPY into LH and NAc, but not VTA, enhanced sucrose consumption in the free-feeding paradigm.

In line with the literature (5), we demonstrate that when fed *ad libitum*, NPY signaling in LH enhances palatable food intake; however, incentive motivation for sucrose remains unaffected. This underlines the role of LH in NPY-mediated hyperphagia, but the downstream pathways remain unknown (5). Although NPY in LH enhanced sucrose free feeding, the 24-h chow intake remained unaltered. As chow intake was measured 24 h after NPY treatment, any orexigenic effects of NPY at an earlier time point cannot be excluded.

NPY receptors are expressed on orexin- and MCH-containing neurons in the LH (10,11). These are orexigenic neuropeptides that act downstream of the leptin-sensitive NPY/POMC neurons. Orexin-containing projections from the LH to the VTA are known to modulate motivation for palatable rewards (12) without affecting free feeding (13). As infusion of NPY into LH failed to enhance motivation for sucrose, it is unlikely that intra-LH NPY activated this population of orexin-containing neurons to the VTA. Interestingly, both orexin and MCH neurons project to the NAc and infusion of these peptides within the NAc provokes food consumption (14,15), implicating a role for LH–NAc connections in mediating the effects of NPY on sucrose free feeding.

Our current findings are supported by the fact that orexin and MCH neurons lie downstream of the arcuate NPY neurons and possibly mediate the orexigenic effects of NPY. However, studies have also shown that applied *ex vivo*, NPY inhibits orexin and MCH neurons (10,11). This evidence and the fact that multiple NPY receptor subtypes are expressed within the LH (both post- and presynaptically), calls for neuronal projection-specific studies as a next step to unravel the role of NPY in the LH.

Similar to the NAc, infusion of NPY into the VTA increased motivation for palatable food. However, free feeding remained unaffected, indicating an exclusively motivational role of NPY within the VTA. Given the role of mesolimbic dopamine in the motivation for food (16,17), it is conceivable that the effect of intra-VTA NPY on responding for sucrose was mediated by dopaminergic neurotransmission. Central NPY administration increases striatal dopamine levels, especially within the NAc, an effect mediated by Y5 receptors (9). As (a) NPY receptors are expressed on the dopaminergic neurons in the VTA (8) and (b) that increased NAc dopamine levels are associated with enhanced incentive motivation (18), we hypothesized that administration of NPY into VTA potentiates dopamine release and enhances motivation. Indeed, the augmented motivation for sucrose observed upon infusion of NPY into VTA was attenuated after pretreatment with a dopamine receptor antagonist, supporting the role of dopamine in NPY-induced motivation for sucrose.

Interestingly, infusion of NPY into the VTA increased the motivation for sucrose but not its intake. Thus, after intra-VTA NPY infusion, animals were willing to work for a sugar reward but did not consume more when it was provided *ad libitum*. These results are

reminiscent of the previous study, implicating dopamine in motivation for food, rather than in consumption (16,17,19,20).

Infusion of NPY into the NAc augmented free feeding, and it also increased operant responding for sucrose. The effect of NPY within the NAc is therefore both on consumption of and motivation for sugar. Our data are in line with the findings of Josselyn and Beninger (1993), showing the effects of NPY within the NAc on conditioned place preference. In addition, they provide evidence that these effects are dopamine dependent.

The NAc can be considered a critical hub connecting limbic, cortical, and hypothalamic circuits regulating food intake. As the NAc mediates both increased motivation and free feeding, one possibility is that NPY signaling in the NAc induces these separate effects via different neuronal circuits (e.g., ventral pallidum vs. LH). Alternatively, the increase in motivation for sucrose may be secondary to an increase in the positive subjective value ("pleasure") of sucrose, which may result in both an increase in intake and the motivation to respond.

The data presented support the notion of region-specific diversity of NPY action on food-directed behavior. In addition to the earlier studies demonstrating the motivational and hyperphagic effects of central NPY infusion, we identified specific neural substrates underlying NPY-enhanced operant responding for sucrose. Our studies further identify NPY–dopamine interaction at the level of the VTA as a key player for NPY-driven motivation for food rewards. O

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