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Neuropeptide Y injected in the paraventricular hypothalamus: A powerful stimulant of feeding behavior

(drinking behavior/norepinephrine/phentolamine)

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ABSTRACT **Neuropeptide Y (NPY) was injected directly into the paraventricular nucleus of the hypothalamus (PVN) of satiated, brain-cannulated rats, and food and water intake were measured 0.5, 1, 2, 4, and 22 hr postinjection. NPY (24, 78, 235, 783, and 2351 pmol/0.3 μl) produced a large, dose-dependent increase in food intake as well as a small increase in water intake. The latency to eat was about 10 min, with substantial feeding occurring in the first 30 min. At doses below 78 pmol, the eating generally occurred only within the first hour. At doses above 235 pmol, however, the subjects' food intake continued to increase such that by 4 hr postinjection they had consumed the equivalent of normal 22-hr intake, and 22 hr postinjection they had also eaten significantly more than control subjects. Previous studies have shown that norepinephrine injected into the PVN stimulates feeding through α-adrenergic receptors. To investigate a possible interaction, subjects were given PVN injections of phentolamine (60 nmol) prior to injections of either NPY (78 pmol) or norepinephrine (20 nmol). Phentolamine pretreatment significantly decreased feeding elicited by norepinephrine without affecting feeding elicited by NPY. This suggests that NPY does not stimulate feeding through the release of endogenous norepinephrine. The powerful stimulation of feeding elicited by this neuropeptide suggests an important role for hypothalamic NPY, or a structurally related peptide, in the regulation of feeding behavior.**

Neuropeptide Y (NPY), a 36-amino acid member of the pancreatic polypeptide family, was discovered in porcine brain by Tatemoto *et al.* in 1982 (1, 2). Subsequently, the concentration of NPY in the brain was found to exceed the concentration of any other peptide (3). Our interest in a possible role for NPY in the control of feeding behavior was stimulated by the recent reports that it is colocalized with norepinephrine (NE) and epinephrine in brainstem catecholamine cell groups, which send afferent fibers to the paraventricular nucleus of the hypothalamus (PVN) (4, 5; however, see ref. 32), and also by reports that it is found within numerous presynaptic terminals in the PVN (3, 6, 7).

Experiments investigating brain mechanisms of feeding behavior have demonstrated that central injections of several putative neurotransmitters, including NE, γ-aminobutyric acid, acetylcholine, and opiate agonists, can selectively stimulate feeding behavior (8–11). The most intensely studied of these is NE. It has been shown that PVN injection of exogenous NE, or drug-induced release of endogenous NE from brainstem catecholamine neurons that ascend to the PVN, elicits feeding behavior through stimulation of α_2-adrenergic receptors (12–15). The feeding response appears to be mediated through the PVN, since this is the brain region that is most sensitive to NE and since NE-induced feeding is severely attenuated by PVN lesions (12, 16). A role for this system in natural (deprivation-induced) feeding was suggested by the findings that PVN α-adrenergic receptors are down-regulated during fasting and are normalized after eating and by the finding that medial hypothalamic release of NE occurs during spontaneous feeding (17, 18).

These findings, in conjunction with the localization of NPY in NE neurons and in terminals of the PVN, led us to investigate a role for NPY in feeding. Recently we demonstrated that, like NE, PVN injection of NPY elicits a feeding response in satiated rats (19). Intracerebroventricular injection of NPY has also been shown to elicit feeding behavior (20, 21). The present study demonstrates that injection of NPY into the PVN elicits the most powerful feeding response obtained to date by chemical injection and also investigates a possible functional interaction of NPY and NE in this effect.

MATERIALS AND METHODS

Subjects and Surgery. Twenty-four adult male Sprague–Dawley rats, weighing 330–450 g at the time of surgery, were housed in a temperature-controlled (22°C) colony room, with a 12:12-hr light/dark cycle with lights on at 7:00 A.M. Subjects were stereotaxically implanted, under Metofane anesthesia, with a chronic 26-gauge stainless-steel guide cannula targeted 1.0 mm dorsal to the PVN. With the incisor bar 3.0 mm above the interaural line, the stereotaxic coordinates were 0.4 mm posterior to bregma, 0.4 mm lateral to the midsaggital sinus, and 7.2 mm ventral to the surface of the skull. Subjects were repeatedly handled and mock-injected during the 7-day recovery period to habituate them to the injection procedure.

Procedures. Rats were maintained and tested on a milk-mash diet consisting of 46% Purina Rat Chow, 37% sucrose, and 17% Carnation evaporated milk. To ensure that subjects were fully satiated at the time of the test, they were given freshly prepared diet 1 hr before the test, which generally began 2 hr after light onset. In 10 of the subjects, a dose–response analysis of the feeding effects produced by NPY was conducted. These subjects were injected with NPY (Peninsula Laboratories, Belmont, CA) at doses of 8, 24, 78, 235, 783, and 2351 pmol (0.033, 0.10, 0.33, 1.0, 3.3, and 10.0 μg) or isotonic saline (0.3 μl) in counterbalanced order. The injections were made directly into the PVN through 33-gauge injectors that extended 1.0 mm beyond the guide cannula. Food and water intake were measured 0.5, 1, 2, 4, and 22 hr postinjection.

In light of the close association between NE and NPY (4, 22), the impact of the α-adrenergic antagonist phentolamine on feeding elicited by NPY and NE was tested. Fourteen satiated subjects were given PVN injections of phentolamine

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Abbreviations: NPY, neuropeptide Y; NE, norepinephrine; PVN, paraventricular nucleus of the hypothalamus.

prior to injections of NPY or NE. First, all subjects were given PVN injections of saline (0.3 μl), and food intake was measured 45 min postinjection. Subsequently, they were given PVN injections of phentolamine (60 nmol/0.3 μl) or its vehicle (0.3 μl of deionized water) in counterbalanced order, followed 15 min later by PVN injections of NE (20 nmol/0.3 μl) or NPY (78 pmol/0.3 μl). Food intake was measured 45 min postinjection.

Histology. Following behavioral testing, all subjects were sacrificed with an overdose of Nembutal and perfused intracardially with isotonic saline followed by 10% formalin. Brains were cut in 100-μm coronal sections and stained with neutral red, and the injection sites were determined with the aid of a stereotaxic atlas (23). The majority of the cannula tracts terminated within or immediately dorsal to the PVN. Those not in the nucleus ranged from 0.3 mm anterior/posterior and 0.3 mm dorsal to the PVN.

Statistics. Data were analyzed by analysis of variance, with multiple comparisons to the control group made by Duncan's new multiple-range test with α equal to 0.05 and 0.01. Single comparisons were made by two-tailed t tests for dependent means.

RESULTS

As shown in Fig. 1, injection of NPY into the PVN produced a strong, dose-dependent increase in food intake [$F(6,54) = 37.1$ $P < 0.001$]. The lowest effective dose was 24 pmol (100 ng) and a dose of 235 pmol was maximally effective. At doses of 78 pmol and above, every subject ate within 60 min postinjection. The mean latency to eat, which did not vary across doses, was 10.2 min with individual subjects ranging from 1 to 37 min. At doses of 24 and 78 pmol, the increase in food intake was essentially confined to the first hour. At higher doses (235 pmol or more), however, the subjects' food intake continued to increase, such that by 4 hr postinjection they had consumed an amount (28 g) approximately equivalent to their normal daily intake (31 g), and, at 22 hr, a significant (at least $P < 0.05$) increase was still apparent.

As shown in Table 1, injection of NPY into the PVN produced a significant increase in water intake [$F(6,54) = 2.6$, $P < 0.05$]. This increase in drinking was small relative to the increase in eating and was not clearly dose-dependent.

As can be seen in Fig. 2, injection of NE (20 nmol) and NPY (78 pmol) into the PVN produced approximately equivalent increases in food intake 45 min postinjection. Pretreatment with phentolamine produced a 67% decrease ($P < 0.01$) in feeding elicited by NE. In contrast, feeding elicited by NPY was unaffected by this α-adrenergic antagonist.

DISCUSSION

This report demonstrates that injection of NPY into the PVN elicits a strong, dose-dependent feeding response, which occurs with a short latency in satiated rats. This eating response appears to be the most dramatic effect obtained to date by either central or peripheral chemical injection. A single injection of NPY in the PVN induced rats to eat in 4 hr what they would normally eat in the 24 hr. This occurred, furthermore, during the daylight hour when feeding behavior is normally very low. With the lower doses of NPY, the feeding response was essentially completed within 1 hr. This contrasts with the higher doses, where feeding continued to increase such that a reliable enhancement of food intake 22 hr postinjection was observed. This finding demonstrates a significant increase in total daily food intake consequent to a single drug injection into the brain. With third ventricular (20) or lateral ventricular (21) injection of NPY, a smaller eating response has been described recently. The present report demonstrates that with PVN injection, doses of NPY 1–2 orders of magnitude lower than used in the ventricles elicit an equivalent feeding response. Furthermore, 1 μg (235 pmol) of NPY in the PVN elicits approximately four times as much eating as 2 μg injected into the third ventricle (20).

Previous studies have shown that intracerebral injection of four neurotransmitters, or their agonists, elicits feeding behavior. They are NE (8), opiates (9), γ-aminobutyric acid (10), acetylcholine (11), and now NPY. Of the first four, NE appears to be most powerful, producing peak intakes of about 8 g 1 hr after injection of 50 nmol (13). By contrast, we report here that 235 pmol of NPY elicits 16 g of food intake 1 hr postinjection and 28 g 4 hr postinjection. This suggests

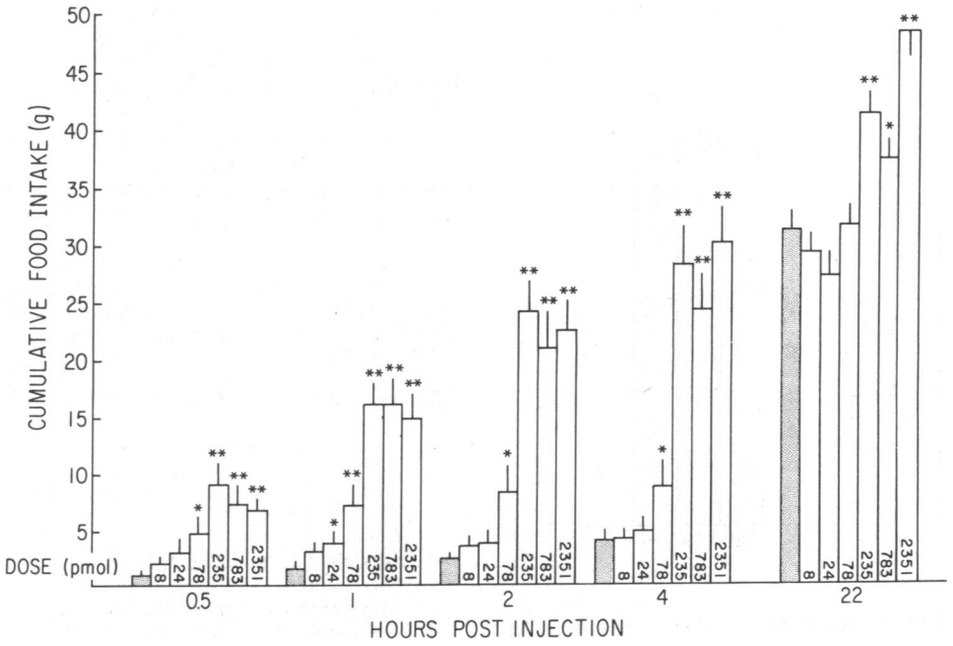

FIG. 1. Cumulative food intake (mean ± SEM) by rats 0.5, 1, 2, 4, and 22 hr following PVN injection of NPY (8, 24, 78, 235, 783, or 2351 pmol/0.3 μl) or vehicle (shaded bar). * = $P < 0.05$ and ** = $P < 0.01$ relative to vehicle by Duncan's new multiple-range test.

Table 1. Effect of PVN NPY injection on water intake at various postinjection intervals

	Water intake, ml						
		NPY, pmol					
Time, hr	Vehicle	8	24	78	235	783	2351
0.5	0.3 ± 0.1	0.7 ± 0.3	0.9 ± 0.3	1.4 ± 0.4**	0.4 ± 0.2	1.1 ± 0.5*	0.3 ± 0.1
1	0.6 ± 0.2	0.9 ± 0.3	1.3 ± 0.4	1.6 ± 0.4*	1.0 ± 0.3	1.2 ± 0.5	0.6 ± 0.1
2	0.7 ± 0.2	1.0 ± 0.3	1.3 ± 0.4	2.3 ± 0.4**	2.0 ± 0.5*	2.1 ± 0.7*	2.3 ± 0.5**
4	1.3 ± 0.5	1.7 ± 0.9	1.3 ± 0.4	3.1 ± 0.9	3.4 ± 0.7*	4.5 ± 1.5**	4.8 ± 1.4**
22	23.5 ± 2.5	21.9 ± 3.0	25.4 ± 3.4	26.1 ± 2.6	25.7 ± 2.1	27.6 ± 3.2	27.4 ± 4.0

Data are presented as mean ± SEM. * = $P < 0.05$ and ** = $P < 0.01$ relative to vehicle by Duncan's new multiple-range test.

that NPY is the most effective chemical stimulator of eating discovered to date and, thus, may play an important functional role in the control of feeding behavior. This hypothesis is supported by our previous finding that the effect of PVN injection of NPY is specific to ingestive behavior (19). With food present, NPY-injected subjects began to eat within 10 min, consumed a single meal, and exhibited a sequence of behaviors previously characterized as the postprandial satiety sequence (24). In contrast to the strong effect on feeding, there was no effect on any noningestive behavior or on levels of activity. This hypothesis is also supported by the recent report that the effect of NPY on feeding is site specific, confined to hypothalamic as opposed to extrahypothalamic sites (25).

This report also demonstrates that injection of NPY into the PVN elicits a small, variable drinking response that was not clearly dose-dependent. Clark *et al.* (20) observed a similar increase in time spent drinking following third ventricular NPY injection. This response appears to be a primary effect of NPY rather than secondary to feeding since (*i*) drinking is also elicited in the absence of food, (*ii*) the magnitude of the eating and drinking did not appear to be correlated, and (*iii*) the effects could be partially dissociated by injections into different brain regions (19, 25). These results suggest that NPY may also play a role in the regulation of drinking behavior.

One of the issues addressed in the present study concerned a possible interaction of NPY and NE with respect to feeding behavior. Previous reports from this laboratory have demonstrated that hypothalamic injection of NE elicits feeding behavior in satiated rats, that the PVN is the brain site most sensitive to this effect, and that the response is elicited through activation of α_2-adrenergic receptors (12, 13, 15). Numerous reports suggest a functional interaction between NE and NPY. They appear to be coreleased from sympathetic nerves, apparently acting together to regulate vasoconstriction by a postsynaptic mechanism (22, 26) and muscular contraction through a presynaptic mechanism (27). Parallel effects of the α-adrenergic agonist clonidine and NPY on several physiological parameters have been observed (28, 29). α_2-Adrenergic but not α_1-adrenergic receptors were up-regulated by NPY (30), and the parameters of NPY binding were altered by an adrenergic antagonist (31).

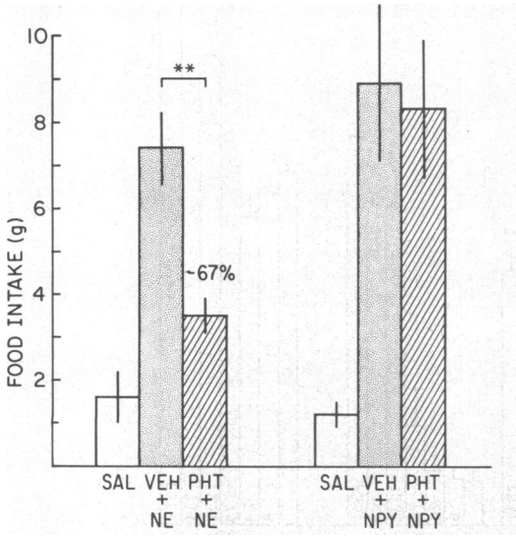

FIG. 2. Effect of PVN phentolamine (PHT) pretreatment on food intake (mean ± SEM) elicited by PVN injection of NE or NPY 45 min postinjection. VEH, vehicle; ** = $P < 0.01$ by two-tailed *t* test for dependent means.

These findings, in conjunction with the high levels of NPY found within the PVN (3, 6, 7) as well as within central NE and epinephrine-containing neurons (4), suggested a possible interaction of NE and NPY that could be important in feeding behavior. The present report, however, demonstrates that blockade of PVN α-adrenergic receptors by phentolamine, which produced substantial decreases in feeding elicited by PVN injection of NE, did not attenuate feeding elicited by NPY. Intracerebroventricular injection of phentolamine also has been shown to be ineffective (21). These results suggest that NPY does not elicit feeding through release of endogenous NE in the PVN, although NPY and NE may still interact at the receptor level. The present findings do not provide evidence supporting a functional interaction of NE and NPY with respect to feeding behavior; however, this issue clearly merits further investigation.

In sum, PVN injection of NPY caused a robust feeding response and a small drinking response in satiated rats. The magnitude of the feeding response, in conjunction with the high concentration of NPY binding sites and immunoreactivity within the hypothalamus (3, 31), suggests an important physiological function for NPY or structurally related peptides in the hypothalamic regulation of feeding behavior.

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