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Evidence for Neuropeptide Y Mediation of Eating Produced by Food Deprivation and for a Variant of the Y_1 Receptor Mediating This Peptide's Effect

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STANLEY, B. G., W. MAGDALIN, A. SEIRAFI, M. M. NGUYEN AND S. F. LEIBOWITZ. *Evidence for neuropeptide Y mediation of eating produced by food deprivation and for a variant of the Y_1 receptor mediating this peptide's effect.* PEPTIDES 13(3) 581–587, 1992.—Neuropeptide Y (NPY) elicits eating when injected directly into the paraventricular nucleus (PVN) or perifornical hypothalamus (PFH). To identify the essential regions of the NPY molecule and the relative contributions of Y_1 and Y_2 receptors, the eating stimulatory potency of NPY was compared to that of its fragments, analogues, and agonists when injected into the PVN or PFH of satiated rats. Additionally, antisera to NPY was injected into the cerebral ventricles (ICV) to determine whether passive immunization suppresses the eating produced by mild food deprivation. Tests with NPY fragments revealed that NPY(2–36) was surprisingly potent, nearly three times more so than intact NPY. In contrast, fragments with further *N*-terminal deletions were progressively less effective or ineffective, as was the free acid form of NPY. Collectively, this suggests that both *N*- and *C*-terminal regions of NPY participate in the stimulation of eating. Tests with agonists revealed that the putative Y_1 agonist [Pro^{34}]NPY elicited a strong dose-dependent feeding response, while the putative Y_2 agonist, C2-NPY, had only a small effect at the highest doses. Although this suggests mediation by Y_1 receptors, the uncharacteristically high potency of NPY(2–36) may additionally suggest that the receptor subtype underlying feeding is distinct from that mediating other responses. Additional results revealed that ICV injection of antisera to NPY, which should inactivate endogenous NPY, produced a concentration-dependent suppression of eating induced by mild food deprivation. This finding, along with published work demonstrating enhanced levels of hypothalamic NPY in food-deprived rats, suggests that endogenous NPY mediates the eating produced by deprivation.

Neuropeptide Y Neuropeptide Y receptors Eating Feeding behavior Paraventricular nucleus
Perifornical hypothalamus Hypothalamus

EVIDENCE suggests that neuropeptide Y (NPY), a member of the structurally related 36 amino acid pancreatic polypeptide family consisting of peptide YY (PYY) and pancreatic polypeptide, is a neurotransmitter or neuromodulator. It is localized primarily, and in extremely high concentrations, within the central and sympathetic nervous systems of many species (2,23), and its receptors are widely distributed in the brain (25). Central administration of NPY produces a variety of physiological, endocrine, and behavioral effects (17), including stimulation of food intake.

Clark et al. first demonstrated that NPY, and to a lesser extent pancreatic polypeptide, injected intracerebroventricularly (ICV) elicits eating in satiated rats (8). We demonstrated that NPY injected directly into the paraventricular hypothalamus (PVN) produces a stronger response at lower doses (42,43), and that the perifornical hypothalamus (PFH) just caudolateral to the PVN is the most sensitive hypothalamic site for this effect (44).

Substantial evidence suggests that hypothalamic NPY has a role in the control of natural eating behavior. It is the most powerful known neurochemical stimulant of eating behavior, producing an eating response that can be near the maximum of rats' physiological capacity (43). Its behavioral effect can also be highly specific (42), and repeated injections of NPY or PYY can produce marked and sustained overeating and obesity (30,41). One of its functions may be to control carbohydrate ingestion, since either acute or repeated injections of NPY preferentially

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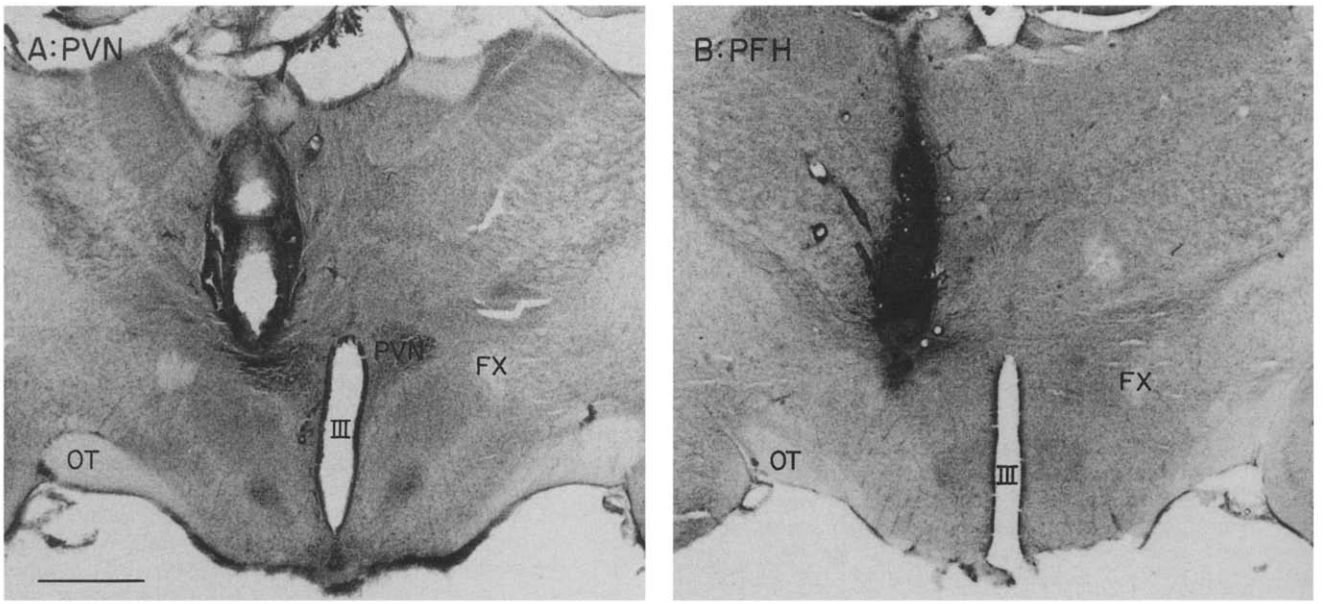

FIG. 1. Frontal sections of rat hypothalamus showing injection sites representative of subjects with cannulas aimed at the PVN (A) or the PFH (B). III, third ventricle; FX, fornix; OT, optic tract; PVN, paraventricular nucleus; bar = 1.0 mm.

enhanced the consumption of this macronutrient (38,40). The activity of hypothalamic NPY may also be altered in normal and pathological conditions accompanied by increased eating. For example, food deprivation, diabetes, and some forms of genetic obesity are associated with increased hypothalamic levels of NPY and NPY gene expression (5,20,35,36,49). Additionally, bulimic patients exhibit markedly higher cerebrospinal fluid levels of PYY while abstaining from binging (4). To establish whether endogenous NPY is essential to the expression of a natural eating response, the present study examined whether eating elicited by mild food deprivation can be inhibited by central administration of NPY antibodies, which are expected to inactivate endogenous NPY.

An additional issue concerns the structure–activity relationship between NPY and eating. In almost all biological systems, the full expression of NPY's effects depends upon an intact *C*-terminal; deletion of a single amino acid can abolish NPY's biological effects (47). With respect to the *N*-terminal, amino acid deletions produce at least two patterns. In some systems successive deletions result in sharp (i.e., orders of magnitude) declines in responsiveness. Other systems exhibit minimal declines, approximately an order of magnitude for fragments with about 12 *N*-terminal deletions [i.e., NPY(13–36)]. These two patterns are believed to reflect the existence of two different NPY receptor subtypes, Y_1 and Y_2, respectively (47). This conclusion was supported by studies with NPY agonists specific for these receptor subtypes (12,16). Therefore, additional goals of the present study were to use NPY fragments to reveal which aspects of NPY's structure are important for stimulation of eating, and selective agonists to determine which receptor subtype mediates its effect. The initial fragment studies used the PVN as the injection site. However, subsequent tests with selective agonists used the PFH as the injection site, based on the recent evidence that this region is more sensitive to NPY's stimulatory effect on eating (44). Portions of this work have been presented in preliminary form (24), and some of the effects we reported have been observed by others (18,19,21,27).

METHOD

Subjects and Surgery

Adult male Sprague–Dawley rats were obtained either from Charles River Labs, Inc. (n = 70; 51 were tested with NPY fragments and 19 were tested with NPY antisera) or from Simonsen Labs (n = 17, tested with NPY receptor agonists). (This change in vendors was due to relocation by the senior author and is noted because Sprague–Dawley rats from Simonsen are found to be less responsive to NPY than those from Charles River.) The subjects were individually housed in hanging stainless steel cages in a vivarium on a 12/12 h light/dark cycle. Animals weighing 330–400 g were stereotaxically implanted, under Metofane anesthesia, with a 26-gauge stainless steel guide cannula targeted 1 mm dorsal to either the PVN (n = 51), the PFH (n = 17), the third cerebroventricle (n = 11), or the lateral cerebroventricle (n = 8). The stereotaxic coordinates for the: a) PVN were 6.3 mm anterior to the interaural line, 0.4 mm lateral to the midsagittal sinus, and 7.2 mm ventral to the surface of the skull, with the incisor bar at +3.0 mm; b) PFH were 7.2 mm anterior, 1.0 mm lateral, and 7.7 mm ventral, with the incisor at −3.3 mm; c) third ventricle were 6.8 mm anterior, 0.0 mm lateral, 7.6 mm ventral, with the incisor bar at +3.0 mm; and d) lateral ventricle were 0.7 mm anterior to Bregma, 1.3 mm lateral, and 4.0 mm ventral, with the incisor at +3.5 mm. The animals were allowed at least 7 days to recover, during which they were repeatedly handled and mock-injected to adapt them to the testing procedures.

Tests With NPY Fragments and Y_1 or Y_2 Receptor Agonists

Following recovery, subjects were tested with NPY (78 pmol) and vehicle to ensure that they exhibited a feeding response of at least 3 g within 60 min of NPY injection. The 10–20% of the subjects that did not were eliminated, and the remaining 68 were divided, based on their elicited eating scores, so as to yield six closely matched groups. Matching was accomplished by rank

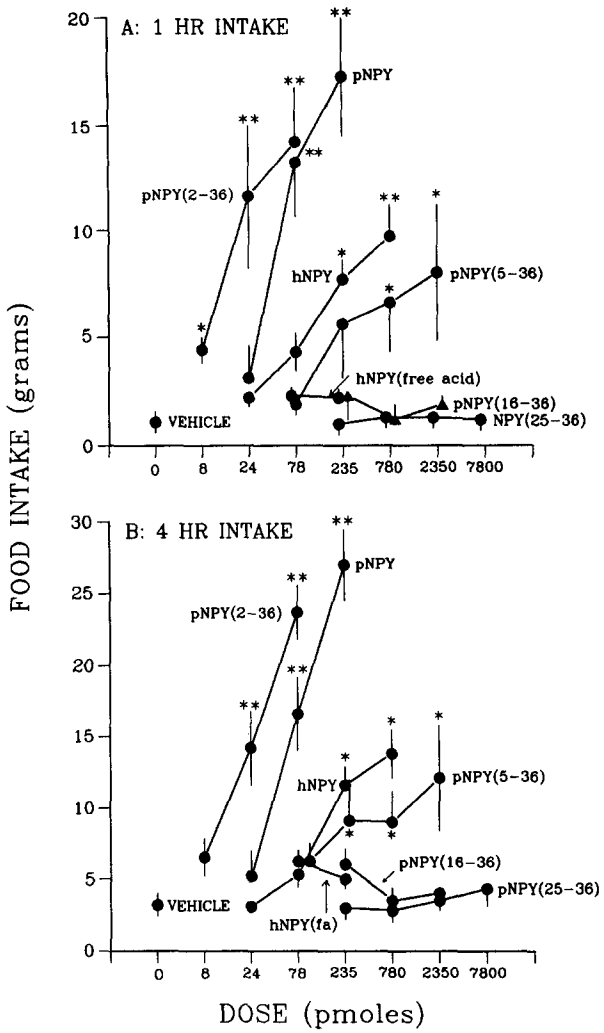

FIG. 2. Effects of PVN injection of pmol doses of NPY and its fragments on cumulative food intake 1 h (A) and 4 h (B) postinjection. Values are mean grams ± SEM. For clarity, the symbols for pNPY(16-36) at 1 h postinjection are triangles, and at 4 h postinjection the label for hNPY free acid is shortened to hNPY(fa). *, $p < 0.05$, **, $p < 0.01$ relative to vehicle by Duncan's multiple range test.

ordering the animals from highest to lowest and then assigning successively ranked subjects to different groups. Animals were maintained and tested with ad lib access to water and a sweetened milk-mash diet, consisting of 46% Purina rat chow, 37% sucrose, and 17% Carnation evaporated milk (i.e., 500 g chow, 400 g sucrose, and a 354 ml can of milk). To ensure that the animals were satiated, they were given freshly prepared diet approximately one h before the injection, which was given 4–6 h after the onset of the light phase. Injections were given through a 33-gauge injector which projected 1.0 mm beyond the guide cannula, directly into the PVN or the PFH. Peptides and vehicle [sterile artificial cerebrospinal fluid (aCSF); Na^+ 147 mM, Cl^- 154 mM, K^+ 3.0 mM, Ca^{++} 1.2 mM, and Mg^{++} 0.9 mM, pH = 7.4] were injected in a volume of 0.3 μl, and food intake was measured 1, 2, and 4 h later. Tests were conducted every 2 to 3 days, and each group of rats was tested with one or two of the various compounds, at all doses. The peptides and doses were: porcine NPY (24–235 pmol); pNPY(2–36) (8–78 pmol); pNPY(5–36) (78–2350 pmol); pNPY(16–36) (235–2350 pmol); pNPY(25–36) (78–7800 pmol); human NPY (24–780 pmol); hNPY free acid (78–235 pmol); [Pro^{34}]NPY (24–780 pmol); [Cys^2,8-aminooctanoic $acid^{5-24}$,$DCys^{27}$]NPY (C2-NPY, 24–2350 pmol). (Porcine and human NPY and its fragments were donated by A. Fournier and S. St-Pierre, or were obtained from Peninsula Laboratories, Belmont, CA. Receptor agonists to NPY were donated by J. L. Krstenansky and T. W. Schwartz.)

Tests With Antisera to NPY

Antisera to NPY (#412 from T. L. O'Donohue) was injected into the lateral (n = 8) or third ventricle (n = 11) of 6-h food-deprived rats, and their subsequent food intake was measured. The food deprivation was initiated during the first 2 h of the light phase, and 5 h later the rats were given a 0.9 μl volume ICV injection of either 100%, 10%, or 1.0% NPY antisera, or preimmune sera, which was used to dilute the NPY antisera. Approximately 1 h later, after a total of 6 h of food deprivation, food was returned, and intake was measured 1 and 2 h later.

Histology and Statistics

The animals were sacrificed by CO_2 inhalation and perfused transcardially with 10% formalin. Brains were removed and 100-μm thick coronal sections were cut through the extent of the cannula track. The sections were stained with cresyl violet and examined microscopically. The location of the injections was determined by tracing a projection of the scar made by the injector onto the atlas of Paxinos and Watson (32).

The data from each peptide was analyzed by a two-way repeated measures ANOVA, followed by Duncan's multiple range tests at $p < 0.05$ or $p < 0.01$. For comparisons of potency, the doses required to produce the equivalent of 50% of NPY's maximum effect (ED_{50}) in the PVN (for fragments and analogues) or PFH (for agonists) were calculated from food intakes 2 h postinjection.

RESULTS

Histology

Histological analysis revealed that the actual injections were located in, or close to, their intended targets. For example, 37 of the 51 PVN animals (73%) had injections within this nucleus, and the remainder were less than 0.4 mm from its borders. Similarly, the PFH animals had injections that were either adjacent to or within 0.3 mm of the fornix. Figure 1 shows representative PVN (A) and PFH (B) sections. Cannulas targeted toward the third and lateral ventricle also were appropriately located.

NPY Fragments

As shown in Fig. 2A and B and reported earlier (43), NPY (24–235 pmol) injected into the PVN produced a strong dose-dependent stimulation of feeding behavior. The drug, time, and interaction effects were, respectively: $F(2, 19) = 14.1$, $p < 0.001$; $F(2, 38) = 87.9$, $p < 0.001$; $F(4, 38) = 17.8$, $p < 0.001$. As shown in Fig. 2A and B, deletion of the first amino acid residue [NPY(2–36)] increased NPY's effectiveness. Thus, this fragment elicited significant eating at a dose of 8 pmol, whereas NPY itself did not produce a significant effect at three times this dose (24 pmol). As shown in Table 1, the ED_{50} decreased from 51.8 pmol for NPY to 18.5 pmol for the 2–36 fragment, a 2.8-fold increase in potency. In contrast, as shown in Fig. 2A,B and in Table 1, deletion of additional amino acid residues led to a marked re-

TABLE 1

AMINO ACID SEQUENCES AND EATING STIMULATORY POTENCY

	ED_{50} (pmol)	Potency Relative to NPY	Sequence
			1 5 10 15 20 25 30 36
pNPY	51.8	1	Y P S K P D N P G E D A P A E D L A R Y Y S A L R H Y I N L I T R Q R Y-NH_2
pNPY(2–36)	18.5	+2.8	P S K P D N P G E D A P A E D L A R Y Y S A L R H Y I N L I T R Q R Y-NH_2
pNPY(5–36)	10,233	−197	P D N P G E D A P A E D L A R Y Y S A L R H Y I N L I T R Q R Y-NH_2
pNPY(16–36)	>50,000	−965	D L A R Y Y S A L R H Y I N L I T R Q R Y-NH_2
pNPY(25–36)	>50,000	−965	R H Y I N L I T R Q R Y-NH_2
hNPY	500	−9.7	Y P S K P D N P G E D A P A E D [M] A R Y Y S A L R H Y I N L I T R Q R Y-NH_2
hNPY free acid	NE	NE	Y P S K P D N P G E D A P A E D [M] A R Y Y S A L R H Y I N L I T R Q R Y [ ]
[Pro^{34}]NPY	216.3	−4.2	Y P S K P D N P G E D A P A E D L A R Y Y S A L R H Y I N L I T R [P] R Y-NH_2
C2-NPY	9,964	−192	Y [C] S K---------------------------- Y S A [c] R H Y I N L I T R Q R Y-NH_2

The dashed line within C2-NPY represents the residues replaced by a single 8-aminooctanoic acid residue. Residues that differ from NPY are boxed. NE = no effect. Abbreviations for the residues are: A = alanine; C = cystine; c = D-cystine; D = aspartate; E = glutamate; G = glycine; H = histidine; I = isoleucine; K = lysine; L = leucine; M = methionine; N = asparagine; P = proline; Q = glutamine; R = arginine; S = serine; T = threonine; Y = tyrosine. ED_{50} represents the total quantity of each peptide, in pmoles, required to yield 50% of the maximum food intake produced by NPY.

duction in the response for NPY(5–36), and to a loss of response for NPY(16–36) and NPY(25–36). More specifically, the ED_{50} of 10,233 pmol for NPY(5–36) represents a 197-fold reduction in potency as compared to pNPY. Additionally, as shown in Fig. 2A,B and in Table 1, human NPY was 9.7-fold less potent than porcine NPY and the free acid form of this peptide was ineffective.

Y_1- Versus Y_2-Selective Agonists

Tests employing specific agonists for different NPY receptor subtypes suggest that a Y_1-like receptor is primarily responsible for the stimulation of feeding. As shown in Fig. 3A,B and Table 1, the putative Y_1 receptor agonist [Pro^{34}]NPY produced a strong eating response, $F(4, 47) = 12.9$, $p < 0.001$. The ED_{50} for this peptide, 216.3 pmol, represents only a 4.2-fold reduction in potency as compared to pNPY. In contrast, the putative Y_2 receptor agonist C2-NPY, while producing statistically significant eating, $F(5, 49) = 3.7$, $p < 0.01$, had an ED_{50} of 9964 pmol, a 192-fold reduction in potency.

NPY Antisera

Tests employing antisera to NPY demonstrate that food deprivation-induced eating can be attenuated by this treatment. As shown in Fig. 4, lateral and third ventricular injections produced a statistically significant suppression of eating for 1 to 2 h [antisera effect was $F(3, 42) = 18.0$, $p < 0.01$ for the lateral ventricle; and $F(3, 60) = 3.1$, $p < 0.05$ for the third ventricle]. (Previous comparisons of lateral and third ventricle effects, by general linear model ANOVA, revealed no significant difference between these sites at $p > 0.4$.) The effects of the antisera were concentration dependent, with 10% and 100%, but not 1%, mixtures significantly suppressing eating, as compared to preimmune sera (0% antisera concentrations). Moreover, the magnitude of the effect of 100% NPY antisera was large, reaching a 60% suppression of vehicle scores for 1-h intake measurements after lateral ventricular injection.

DISCUSSION

The objectives of this study were to determine:

1. the contributions of different aspects of NPY's structure to its feeding-stimulatory effect;
2. the role of Y_1 and Y_2 receptors in this response; and
3. the importance of endogenous NPY activity in the eating response provoked by food deprivation.

In relation to the first goal, alterations of either terminal region modified NPY's potency, suggesting that both the *C*- and the *N*-terminals contribute to NPY-elicited eating. Specifically, deletion of the *N*-terminal tyrosine [pNPY(2–36)] increased potency, while further *N*-terminal deletions markedly reduced or abolished the effect. Also, substitution of the number 17 leucine amino acid of pNPY with methionine to generate hNPY reduced NPY's potency, while modifying the *C*-terminal by desamidation rendered this peptide ineffective. In relation to the second goal, the markedly greater effectiveness of the Y_1 agonist [Pro^{34}]NPY than the Y_2 agonist C2-NPY suggests that the elicited eating is mediated primarily by Y_1 receptors. However, the increased potency of NPY(2–36), which is uncharacteristic of Y_1-mediated effects, suggests that the receptor underlying eating may be a variant of this subtype. In relation to the final goal, the suppression by NPY antisera of feeding induced by brief food deprivation suggests that increased release of endogenous NPY may mediate the eating produced by this manipulation.

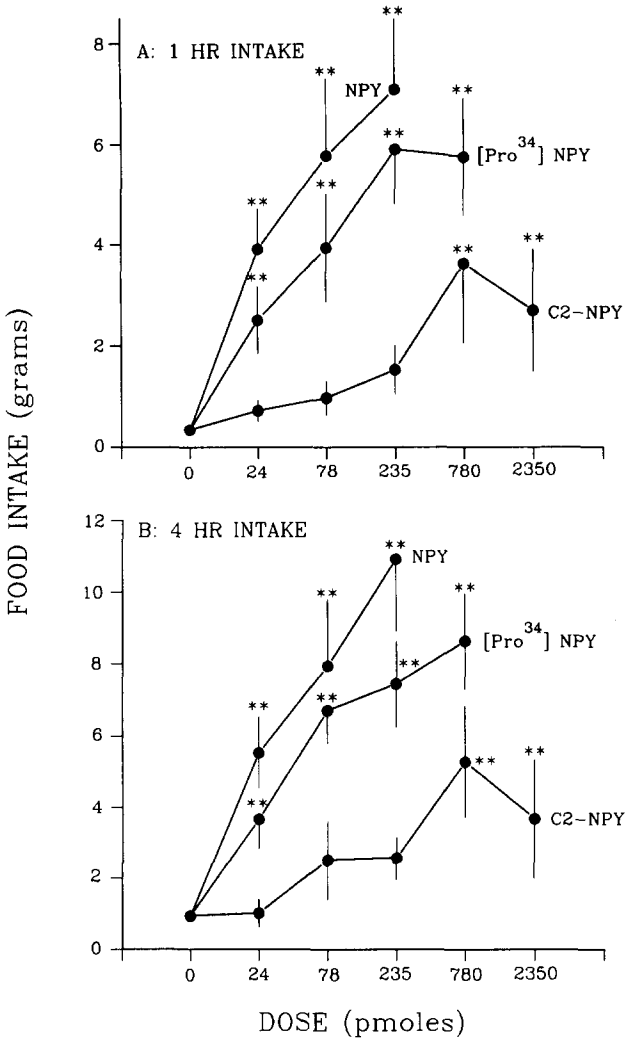

FIG. 3. Effects of PFH injection of pmol doses of NPY, the Y_1 agonist [Pro^{34}]NPY, and the Y_2 agonist C2-NPY on food intake 1 h (A) and 4 h (B) postinjection. Values are mean grams ± SEM. **, $p < 0.01$ relative to vehicle by Duncan's multiple range test.

Except for the enhanced potency of pNPY(2–36) relative to pNPY, the pattern of NPY fragment effects on eating behavior corresponds with their effects in other physiological systems. In other systems both the *N*- and *C*-terminals contribute to NPY's activity, with the *C*-terminal amide essential to almost all of NPY's effects. For example, desamido NPY is ineffective in receptor binding, in electrophysiological preparations, and in several endocrine and autonomic systems (7,10,46,47). It is also ineffective in eliciting eating when injected ICV (28). Likewise, we show that free acid NPY does not elicit eating when injected into the PVN. The elimination of the response by this minor structural change argues that the effect is highly dependent upon NPY's precise structural conformation, and is thus most likely mediated by specific receptors rather than by changes in nonspecific parameters, such as osmotic pressure. In particular, since desamido NPY is altered only at the *C*-terminal, its ineffectiveness argues that this terminal is critical to eating elicited by PVN injection. However, ICV injection of NPY(1–27) (31), but not NPY(1–12) and NPY(1–24) (13,18), has recently been shown to stimulate eating. Therefore, long *N*-terminal fragments of NPY may be sufficient to elicit eating under certain conditions.

Additionally, we show that successive deletion of *N*-terminal amino acids caused marked, progressive declines in elicited eating, suggesting that the *N*-terminal also participates in generating the eating response. Again, this pattern is characteristic of other NPY-induced effects and has been seen in a broad variety of biological preparations (10,13,14,18,34), including the stimulation of food intake (18,19,27). This similarity implies that exogenous NPY elicits eating through a specific action on NPY receptors. In conjunction with the evidence that NPY can produce dramatic overeating and obesity (41,43), this argues for the existence of hypothalamic NPY receptors that can exert an overpowering influence over neural control mechanisms of eating behavior.

As described earlier, there are two different response profiles to NPY fragments with shortened *N*-terminals. Responses where potency declines dramatically are believed to be mediated by Y_1 receptors, while those exhibiting small declines are mediated by Y_2 receptors (47). Of these, the marked loss in eating stimulatory potency exhibited by fragments, like NPY(5–36), that were missing only a few *N*-terminal amino acids is characteristic of Y_1 receptor-mediated effects. Therefore, the fragment work suggests that the Y_1 rather than the Y_2 receptors mediate the eating response elicited by PVN injection of NPY, a conclusion supported by other fragment studies employing ICV and PVN injections (15,18,19,21,27). A role for Y_1 receptors is consistent with the finding that PVN, like ICV (9,13), injection of hNPY (which has a central region amino acid substitution) was less effective than pNPY in eliciting eating. This is based on evidence that Y_1 but not Y_2 receptor effects are sensitive to central region amino acid substitutions that preserve the spatial relationship between the *C*- and *N*-terminal (12,37).

The most direct evidence for Y_1 mediation was provided by PFH injections of the Y_1 agonist [Pro^{34}]NPY and the Y_2 agonist C2-NPY (12,16,37). Specifically, [Pro^{34}]NPY elicited a dose-dependent feeding response only slightly smaller than that produced by NPY itself. Kalra et al. (19) and Leibowitz et al. (21) have also observed eating in response to either PVN or ICV injections of a similar Y_1 agonist, [Leu^{31},Pro^{34}]NPY. The similar

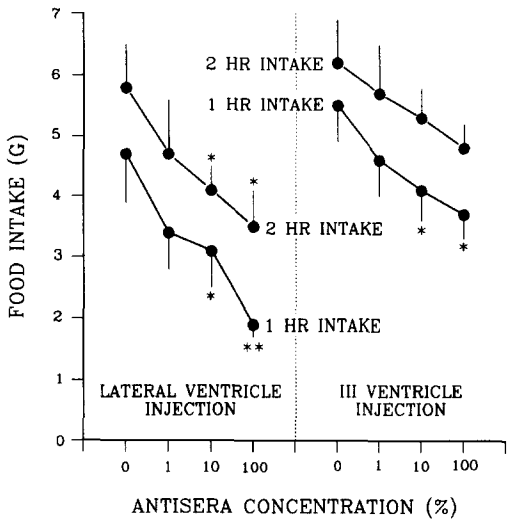

FIG. 4. Suppressive effects of lateral and third ventricular administration of increasing concentrations of NPY antisera on the food intake (mean grams ± SEM 1 and 2 h postinjection) of rats food deprived for 6 h. *, $p < 0.05$, **, $p < 0.01$ relative to vehicle scores at the same postinjection interval by Duncan's multiple range test.

effects of PVN and PFH injection, in addition to supporting a role for Y_1 receptors, suggest that the receptor types mediating NPY-induced eating may be similar in these two hypothalamic regions. Mediation by the Y_1 receptor is also consistent with the minimal effect of PFH C2-NPY injection observed in the present study, and with the ineffectiveness of NPY(13–36), a Y_2 receptor activating fragment, observed by others (13,19,21). However, the Y_2 receptor also may play a role since C2-NPY did produce a statistically significant eating response.

Collectively, findings with NPY receptor agonists and fragments argue that hypothalamic Y_1 receptors have a central role in the eating response elicited by NPY. There is, however, a finding which suggests that these receptors may be different from those mediating other Y_1 responses. We found that, in the PVN, NPY(2–36) was 2.8 times more potent than NPY in eliciting eating (24), an effect observed by others (19,21). In contrast, with ICV injections, some have reported that NPY(2–36) is more effective than NPY (18,19), while others have reported that it is equal to or even less effective (27). Although these discrepancies will need to be resolved, in the PVN at least, NPY(2–36) is consistently more effective than NPY in eliciting eating. This was unexpected, since earlier studies in other systems found that NPY(2–36) was, at most, as effective as the intact molecule (10), and more frequently was three- to twelvefold less effective (1,6,14,18,34). While the functional significance of these differences cannot be ascertained with confidence at present, the provocative interpretation is that the receptors mediating hypothalamic NPY's eating stimulatory effect are a new variant of the Y_1 subtype. Other findings may be congruent with this suggestion. One is that ICV injections of pancreatic polypeptide elicit eating (8,13) but not most other effects produced by NPY (7,37), suggesting that the receptors mediating feeding and the other effects are distinctive. Also, a comparison of NPY to PYY binding across brain areas revealed that in the PFH/lateral hypothalamus the relationship between the binding strength of these ligands was distinct from that in other brain areas (33), suggesting that the binding characteristics of receptors in the brain site most sensitive to NPY's eating stimulatory effect are different from those in other brain sites.

Another key question is, does endogenous NPY have a role in generating naturally occurring eating behavior? In the present study, this was approached by injecting NPY antisera ICV into mildly food-deprived rats. Antisera to NPY produced a strong concentration-dependent suppression of eating. To our knowledge, this is the first evidence for a suppression of eating by a manipulation that should inhibit the activity of endogenous NPY. This finding, combined with earlier reports that NPY injection elicits eating (8,42,43) and that levels of endogenous NPY and NPY gene expression are sensitive to various manipulations associated with over- and undereating (5,35,36,48,49), argues that endogenous NPY has a role in the control of naturally occurring eating behavior.

In particular, this finding suggests that endogenous NPY, along with other neurotransmitters, may generate the eating produced by food deprivation. Substantial evidence is consistent with this possibility. For example, food deprivation has been shown to elevate PVN levels of tissue NPY, an effect that is reversed by refeeding (3,35). Recently, push-pull cannula measurements have shown that extracellular concentrations of NPY also increase in the medial hypothalamus during food deprivation, suggesting that NPY release is increased in this condition (20). Additional evidence that hypothalamic NPY mediates the eating induced by food deprivation are the similar eating patterns following 24 h of food deprivation and PFH NPY injection [but not ICV NPY injection (22)]. Following either deprivation or PFH injection, subjects eat an enormous initial meal followed by a series of smaller meals that are still abnormally large (45). Thus, a convergence of evidence now suggests that hypothalamic NPY is released in response to food deprivation and participates in generating the consequent eating response. Additionally, NPY is likely to participate in producing the eating occurring in other situations, as suggested by the variety of feeding-related manipulations that appear to influence the activity of hypothalamic NPY (5,35,36,48,49).

However, additional work is needed to establish the significance of the antisera suppression of eating. For example, the anatomical locus of the antisera effect is unknown. While the PFH is the hypothalamic site that is most sensitive to NPY's eating stimulatory effects (44), NPY and related peptides can also act in other hypothalamic areas (29,39), and at brainstem and cortical sites to stimulate eating (11,26). Additionally, it has not yet been demonstrated that the inactivation of endogenous NPY is critical to the effectiveness of the antisera, nor that its effect is behaviorally specific. To address these concerns fully will require the development of high-affinity antagonists that are specific to the receptor subtype mediating NPY's effects on eating. Nevertheless, the present results may represent an important first step that could not only help to clarify the role of NPY in control of eating behavior, but may also assist in the eventual discovery of treatments for disorders such as bulimia, anorexia nervosa, and obesity.

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