[D-TRP^{32}]Neuropeptide Y: A Competitive Antagonist of NPY in Rat Hypothalamus

A. Balasubramaniam,*,† S. Sheriff,† M. E. Johnson,§ M. Prabhakaran,§ Y. Huang,† J. E. Fischer,† and W. T. Chance†,‡

Division of Gastrointestinal Hormones, Department of Surgery, University of Cincinnati Medical Center, and VAMC, Cincinnati, Ohio 45267-0558, and Center for Pharmaceutical Biotechnology and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612-7230

Received September 29, 1993•

Neuropeptide Y (NPY) is a potent orexigenic peptide. Structure–activity studies have revealed that nearly the entire sequence of NPY is required to elicit feeding responses. Therefore, in order to develop antagonistic peptides for NPY-induced feeding, we synthesized full-length analogs of NPY, substituting D-Trp in the C-terminal receptor binding region, and screened their activity in rat hypothalamus. Although [D-Trp^{36}]NPY and [D-Trp^{34}]NPY inhibited isoproterenol-stimulated hypothalamic membrane adenylate cyclase activity, [D-Trp^{32}]NPY exhibited no intrinsic activity. Furthermore, [D-Trp^{32}]NPY inhibited [^{125}I]NPY binding to rat hypothalamic membranes with a potency comparable to that of NPY. The presence of 30 and 300 nM concentrations of [D-Trp^{32}]NPY shifted the inhibitory dose–response curve of NPY on isoproterenol-stimulated hypothalamic membrane adenylate cyclase activity parallel to the right with comparable K_B values. Moreover, in vivo experiments in rats revealed that [D-Trp^{32}]NPY (10 μg) significantly attenuated the 1-h feeding response induced by NPY (1 μg). Several other substitutions at position 32 including 2-D-Nal resulted in agonist activity, suggesting that there are strict structural requirements to induce the antagonistic property in NPY. These findings confirm that [D-Trp^{32}]NPY is a competitive antagonist of NPY in both in vitro and in vivo systems. Analogs based on [D-Trp^{32}]NPY may have potential clinical application, since NPY has been implicated in the pathophysiology of a number of feeding disorders including obesity, anorexia, and bulimia.

Introduction

The findings that neuropeptide Y (NPY), a 36-residue peptide amide isolated from porcine brain with the sequence, YPSKPDNPGEDAPAEDL ARYYSALRHY-INLITRQRY-NH_2, occurs in higher concentrations in mammalian brain than any other peptides isolated to date has spawned numerous investigations of its central effects.[1,2] These studies have shown that microinjection of NPY into various hypothalamic sites can induce robust feeding behavior in rats.[3-5] Investigations in our laboratory suggest that NPY-induced feeding may be mediated by pertussis toxin-sensitive G-proteins in the hypothalamus through a cAMP-dependent pathway.[5] Moreover, localization of NPY in hypothalamic regions known to be involved in the regulation of feeding behavior,[4] together with findings that NPY level and secretion are increased in the hypothalamus of fasted rats and these elevations are normalized with feeding, have led Kalra and co-workers to suggest that NPY may constitute the ultimate neurochemical signal that triggers feeding.[6,7] Therefore, it appears that aberrations in the hypothalamic NPY circuitry may contribute to the pathophysiology of a number of eating disorders such as obesity, bulemia, and anorexia nervosa. Consistently, hypothalamic NPY levels and/or the NPY mRNA have been found to be elevated in hyperphagic obese rats[8] and decreased in anorectic tumor-bearing rats.[9] These observations suggest that the NPY sequence could be modified for therapeutic use.

With this in mind, and because nearly the entire sequence of NPY is required for eliciting feeding responses,[10,11] we synthesized a number of full-length analogs of NPY, substituting D-Trp for Thr^{32}, Gln^{34}, or Tyr^{36} in the C-terminal receptor binding sequence, and investigated their activity in rat hypothalamus. These studies showed that although [D-Trp^{36}]NPY and [D-Trp^{34}]NPY exhibited agonist activity, [D-Trp^{32}]NPY showed no intrinsic agonist activity. [D-Trp^{32}]NPY, however, inhibited the binding of [^{125}I]NPY to hypothalamic membranes with high affinity. We therefore tested [D-Trp^{32}]NPY for antagonistic properties and in this paper provide evidence to show that [D-Trp^{32}]NPY is in fact a competitive antagonist of NPY in rat hypothalamus.

Results

The synthetic peptides were ≥96% homogeneous by analytical reversed-phase chromatography and had the expected amino acid composition and masses.

At 1.0 μM, NPY, [D-Trp^{34}]NPY, [D-Trp^{36}]NPY, and the corresponding formylated D-Trp analogs inhibited the isoproterenol-stimulated hypothalamic adenylate cyclase activity significantly (Figure 1). [D-Trp^{32}]NPY and its formylated derivative, however, did not exhibit a significant inhibitory effect on adenylate cyclase activity at this concentration. In the binding experiments, NPY and [D-Trp^{32}]NPY inhibited ^{125}I-labeled NPY bound to rat hypothalamic membranes in a dose-dependent manner with IC_{50} values of 0.63 and 3.0 nM, respectively (Figure 2, top).

NPY inhibited the isoproterenol-stimulated hypothalamic membrane adenylate cyclase activity dose-dependently with an IC_{50} value 0.18 nM (Figure 2, bottom). [D-Trp^{32}]NPY did not exhibit any inhibitory effect on adenylate cyclase activity. The presence of 30 and 300 nM [D-Trp^{32}]NPY shifted the inhibitory dose–response curve of NPY on hypothalamic adenylate cyclase activity

* Address correspondence to: A. Balasubramaniam, Department of Surgery, University of Cincinnati Medical Center, Cincinnati, OH 45267-0558.
† University of Cincinnati Medical Center.
‡ VAMC.
§ University of Illinois at Chicago.
• Abstract published in *Advance ACS Abstracts*, February 1, 1994.

0022-2623/94/1837-0811$04.50/0

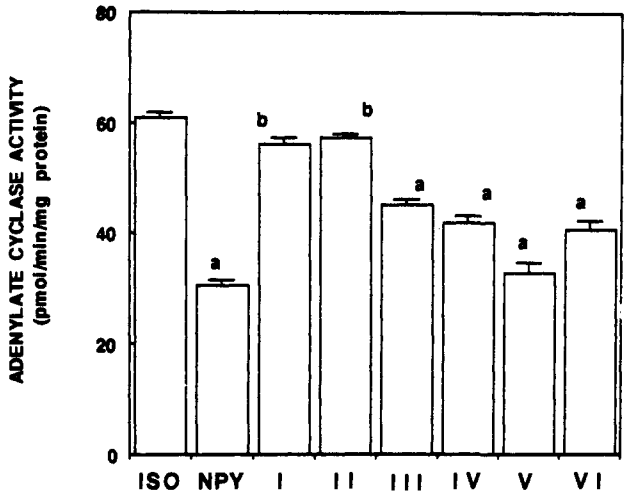

Figure 1. Comparison of the effects of D-Trp- or D-Trp(CHO)-substituted NPY analogs (1.0 μM) on the isoproterenol-stimulated adenylate cyclase activity of rat hypothalamic membranes: (I) [D-Trp^{32}]NPY, (II) [D-Trp(CHO)^{32}]NPY, (III) [D-Trp^{34}]NPY, (IV) [D-Trp(CHO)^{34}]NPY, (V) [D-Trp^{36}]NPY, (VI) [D-Trp-(CHO)^{36}]NPY; a = $p < 0.01$ compared to isoproterenol (by repeated measures of ANOVA); b, not significant compared to isoproterenol.

to the right, increasing the IC_{50} value to 4.0 (K_B = 1.41 nM) and 40.0 nM (K_B = 1.36 nM), respectively (Figure 2, bottom).

To assess the specificity of [D-Trp^{32}]NPY, we investigated its effect on the inhibitory hypothalamic adenylate cyclase activity of serotonin (Figure 3). These experiments revealed that serotonin (100 nM) significantly ($p < 0.01$; by repeated measures ANOVA) inhibited the isoproterenol-stimulated adenylate cyclase activity both in the absence and presence of [D-Trp^{32}]NPY (1 μM).

Since hypothalamic NPY has been shown to elicit a robust feeding response, we also investigated the effect of [D-Trp^{32}]NPY on NPY-induced feeding in freely moving rats. Intrahypothalamic injection of NPY (1 μg) significantly ($p < 0.01$) stimulated the cumulative food intake as compared to vehicle (artificial cerebrospinal fluid) treatment over 1 h (Figure 4). [D-Trp^{32}]NPY (1 μg) did not stimulate feeding significantly over this period, nor did it attenuate NPY (1 μg)-induced feeding at this concentration (not shown). [D-Trp^{32}]NPY (10 μg) also did not exhibit a significant effect on feeding, and at this dose significantly ($p < 0.05$) attenuated the 1 h cumulative food intake induced by 1 μg of NPY (Figure 4).

The effect of replacing D-Trp^{32} with the L-isomer, other aromatic amino acids, and/or residues which might induce similar structural changes were also investigated. In this regard we synthesized [L-Trp^{32}]NPY, [D-Phe^{32}]NPY, [D-Nal^{32}]NPY, [Hyp^{32}]NPY, [3-I-Tyr^{27},D-Trp^{32}]NPY, and [3-I-Tyr^{27,36},D-Trp^{32}]NPY, and studied their effect on isoproterenol-stimulated hypothalamic adenylate cyclase activity. However, all these peptides, at a concentration of 1 μM, significantly inhibited the enzyme activity.

Discussion

NPY exhibits a wide spectrum of central and peripheral activities through a variety of receptor subtypes. At present these receptors have been broadly classified into three main classes, namely Y-1, Y-2, and Y-3 subtypes.[12-14] Although NPY and its homologous peptide, peptide YY (PYY), exhibit nearly equal affinity to both Y-1 and Y-2 receptors, the shorter C-terminal fragments can bind only

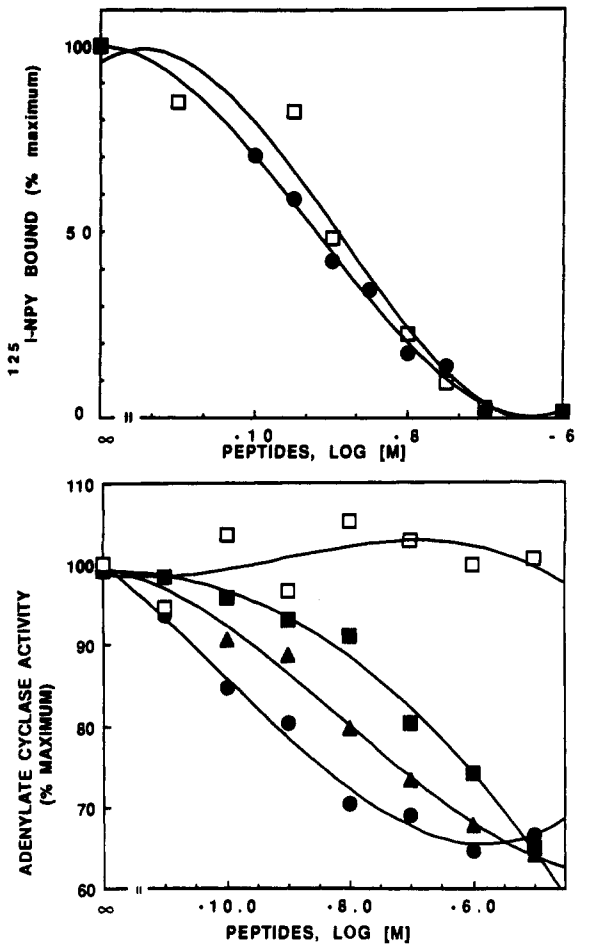

Figure 2. (Top) Displacement of ^{125}I-labeled NPY bound to rat hypothalamic membranes by increasing concentrations of NPY (●) and [D-Trp^{32}]NPY (□). (Bottom) Effects of increasing concentrations of NPY in the absence (●) and the presence of 30 (▲) or 300 nM (■) of [D-Trp^{32}]NPY on the isoproterenol-stimulated adenylate cyclase activity of rat hypothalamic membranes. Also shown is the effect of increasing concentrations of [D-Trp^{32}]NPY (□).

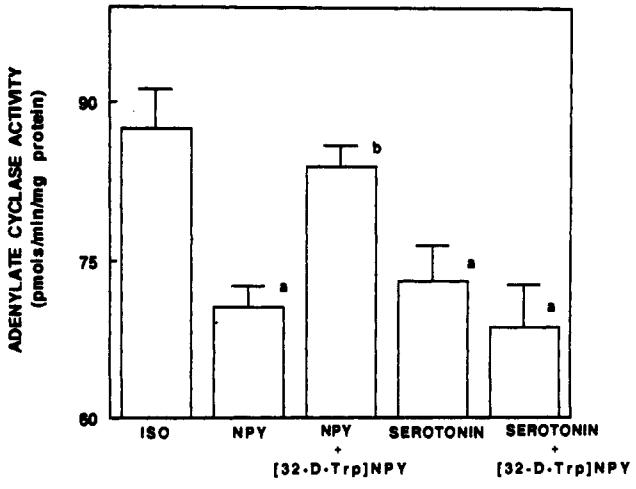

Figure 3. Comparison of the effects of [D-Trp^{32}]NPY (1.0 μM) on the inhibition of isoproterenol-stimulated adenylate cyclase activity of rat hypothalamic membranes by NPY (100 nM) and serotonin (100 nM): a = $p < 0.01$ compared to isoproterenol (by repeated measures of ANOVA); b, not significant compared to isoproterenol.

to Y-2 subtypes. Y-3 receptors, on the other hand, exhibit greater affinity for NPY than PYY.[12,13] However, there is mounting evidence that the NPY receptor system is

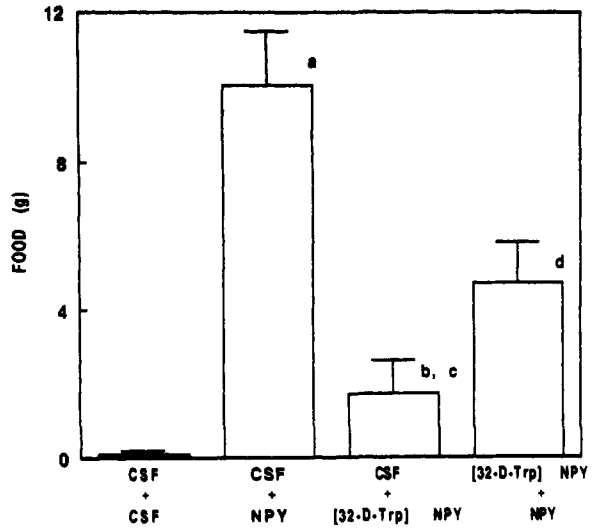

Figure 4. Antagonism of NPY-induced feeding in rats by [D-Trp^{32}]NPY. Groups of rats were pretreated with intrahypothalamic injections (1 μL) of artificial CSF or 10 μg of [D-Trp^{32}]NPY. Fifteen minutes later CSF-treated rats were injected again with CSF (n = 6), 1 μg of NPY (n = 6), or 10 μg of [D-Trp^{32}]NPY (n = 7). The [D-Trp^{32}]NPY-treated rats were injected with 1 μg of NPY (n = 8). Rats were provided with a known quantity of rat chow, and after 1 h the food consumed was determined and corrected for spillage: a = $p < 0.01$ vs CSF (by repeated measures of ANOVA); b, not significant vs CSF; c = $p < 0.01$ vs NPY; d = $p < 0.05$ vs NPY.

much more complex than originally thought, because there may exist further receptor subtypes of NPY such as those in mast cells and heart which exhibit unique properties.[15,16] Therefore, we reasoned that systematic structure–function studies have to be performed with a receptor subtype to delineate the minimum sequence requirements before embarking on designing selective agonist and antagonist peptides for that particular system. This approach has previously led us to identify NPY(17-36) and NPY(18-36) as physiological and competitive antagonists of NPY in rat cardiac ventricular membranes, respectively.[12,16] In the present study designed to develop hypothalamic NPY receptor antagonist, we chose to work with the entire sequence because previous investigations have shown that intact NPY is required to stimulate feeding.[10,11] Furthermore, since the C-terminal region is important for receptor binding, and because D-Trp has generally been found to be a desirable residue to impart antagonistic properties to a number of peptide hormones, including LH-RH[17] and parathyroid hormone,[18] we synthesized analogs substituting D-Trp at position 32, 34, or 36. We did not substitute Arg^{33} or Arg^{35}, because these residues have been shown to be important for interaction with the receptors.[19]

The results presented in this paper demonstrate that although substitution of D-Trp at position 34 or 36 resulted in agonist activity, [D-Trp^{32}]NPY and its formylated derivative were devoid of any intrinsic activity in the hypothalamic membranes. Moreover, [D-Trp^{32}]NPY interacted with hypothalamic membranes with a potency comparable to that of NPY, while the corresponding formylated compound exhibited low affinity (not shown). It is the complete loss of intrinsic activity and the high binding potency that characterized [D-Trp^{32}]NPY as an antagonist of NPY in the hypothalamus. The competitive nature of the antagonism was further confirmed by the finding that two different concentrations of [D-Trp^{32}]NPY shifted the inhibitor dose–response curve of NPY parallel to the right with comparable K_B values. This antagonism was specific to NPY receptors because [D-Trp^{32}]NPY did not have any effect on the inhibitory hypothalamic adenylate cyclase activity of serotonin.

We have shown previously that pertussis toxin treatment of the hypothalamus can block NPY-induced feeding as well as the inhibitory effect of NPY on hypothalamic adenylate cyclase activity.[5] On the basis of these findings, we suggested that hypothalamic cAMP may mediate the effects of NPY on feeding. If this hypothesis is correct, we reasoned that [D-Trp^{32}]NPY should also antagonize NPY-induced feeding. In accordance with this hypothesis, [D-Trp^{32}]NPY significantly attenuated the feeding responses induced by NPY.

With the view of determining the factors inducing antagonistic property in [D-Trp^{32}]NPY, we studied the effects of a number of other substitutions at position 32. A D-residue was found to be essential because L-Trp substitution resulted in agonist activity. Likewise, substitution with other D-aromatic amino acids such D-Phe or even 2-D-Nal, a Trp-mimicking residue, resulted in agonist activity, showing that there are strict structural requirements for exhibiting antagonistic property. While our work was in progress, Tatemoto[20] reported that [3-Cl$_2$-Bzl-Tyr^{27,36},D-Thr^{32}]NPY(27–36) and [3-Cl$_2$Bzl-Tyr^{27},D-Thr^{32}]NPY(27–36) can antagonize NPY-induced intracellular Ca^{2+} mobilization in HEL cells. Therefore, we synthesized similar peptides with D-Trp at position 32. These peptides, [3-I-Tyr^{27},D-Trp^{32}]NPY, [3-I-Tyr^{27,36},D-Trp^{32}]NPY, N-α-Ac-[D-Trp^{32}]NPY(27–36), and [3-I-Tyr^{27,36},D-Trp^{32}]NPY(27–36) were either agonist or had low receptor affinity in the hypothalamus (not shown).

Our finding that a single substitution confers antagonism in NPY is very intriguing, and therefore it is of great interest to determine the structural changes induced by D-Trp substitution. Conformational models based on the X-ray structure co-ordinates of the homologous peptide, avian pancreatic polypeptide (APP),[21] suggest that substitution of D-Trp at position 32 could induce a type II′ β-turn at that position causing the C-terminal to fold back over the PP-fold, rather than extend away from the helix, as it does for the APP crystal structure (Figure 5). Similarly, if the NMR structure is considered, in which the residues 11–36 fold into an amphiphilic helix,[22] the substitution of D-Trp at position 32 should disrupt the helix, potentially producing a similar turn and folding arrangement (not shown). We suggest, therefore, that the introduction of the D-Trp^{32} substitution may induce a type II′ β-turn at this position, and that the modified conformation of this C-terminal region may be responsible for the loss of agonist activity, generating a potent antagonist. In addition, the indole ring appears to be critical because 2-D-Nal substitution failed to impart antagonistic property. Similar structural changes induced by D-Trp substitution in somatostatin[23] and LHRH[17] have previously been exploited toward designing simple and/or potent agonist or antagonistic compounds. Moreover, as in this study, D-Trp could not be replaced by 2-D-Nal in the case of somatostatin analogs.[23] These observations suggest that our findings with [D-Trp^{32}]NPY may also pave the way toward the development of low molecular weight antagonistic compounds which, on peripheral administration, may pass the blood–brain barrier and antagonize NPY-induced feeding.

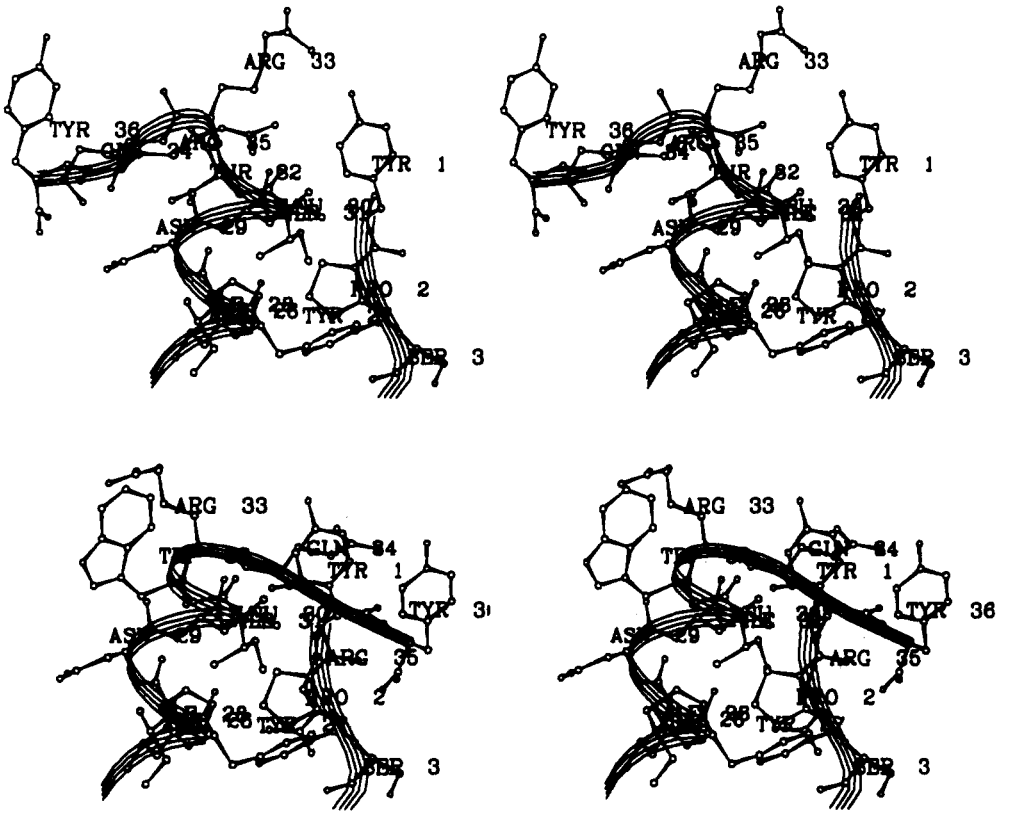

Figure 5. Stereostructure of NPY (top) N- and C-terminal region, based on X-ray structure of avian pancreatic polypeptide as determined by X-ray (see ref 18), with ribbon overlay to emphasize backbone configuration. Model of [D-Trp^{32}]NPY (bottom), with a type II′ β-turn configuration for residues 32 and 33. Note that the proposed model repositions the C-terminal Tyr^{36} into close proximity to Tyr^{1}, potentially providing good aromatic clustering.

Although it is generally believed that the NPY effects on blood pressure and feeding are mediated by the Y-1 receptor subtype, there are reports showing that NPY analogs which elicit pressor effects have no orexigenic effects.[24-26] It appears, therefore, that [D-Trp^{32}]NPY may prove useful not only to elucidate the receptor subtypes mediating NPY effects on hypothalamus, but also to determine whether feeding and pressor effects are mediated by the Y-1 receptors.

In conclusion, we have shown [D-Trp^{32}]NPY to be a competitive antagonist of NPY in the hypothalamus, in both in vitro and in vivo models. [D-Trp^{32}]NPY together with our cardiac NPY receptor antagonist,[12] NPY(18-36), constitute the first generation of antagonists for Y-1 and Y-3 receptors, and provide a framework to develop more potent and selective antagonists for these receptor subtypes. Since NPY has been implicated in the pathophysiology of feeding disorders,[6,8,9] hypertension,[27] and congestive heart failure,[28] the compounds thus developed may prove useful not only in physiological studies but also in developing therapeutic drugs.

Experimental Section

Materials. Protected amino acid derivatives (Midwest Bio-Tech, Indianapolis, IN) and peptide synthesis reagents (Applied Biosystem, Foster City, CA) were obtained commercially and used without further purification. All buffer reagents and protease inhibitors were purchased from Sigma. Sources of rats and other reagents have been described previously.[5,12,13,16]

Peptide Synthesis. Peptides were synthesized using an applied Biosystem Model 430A automated instrument, cleaved by hydrogen fluoride, and purified by reversed-phase chromatography according to our previously published procedures.[29]

Binding and Adenylate Cyclase Studies. The rat hypothalamus was isolated, and the membranes for binding[30] and adenylate cyclase studies[31] were prepared according to the published procedures. Displacement studies were performed in a total volume of 0.25 mL of 20 mM HEPES buffer, pH 7.4, containing 1% bovine serum albumin, 0.1% bacitracin, 300 μM PMSF, and 5 KIU/mL aprotinin. In a standard assay, 100 μg of membrane protein/tube were incubated in a shaking water bath at 24 °C for 45 min with [^{125}I-Tyr^{1}]NPY (~20 000 cpm)[14] in the presence of increasing concentrations of NPY (10^{-11}–10^{-5} M). At the end of incubation, 1.0 mL of ice-cold buffer was added, the mixture was centrifuged at 10000g for 10 min, and the supernatant was removed by aspiration. The tube containing the pellet was counted for bound radioactivity in a micromedic γ-counter.

Adenylate cyclase activity of the hypothalamic membranes was determined by incubating 50 μg of membranes in a total volume of 0.20 mL of Tris-HCl (30 mM, pH 7.4) buffer containing 150 mM NaCl, 8.25 mM $MgCl_2$, 0.75 mM EGTA, 1.5 mM theophylline, 20 μg/mL aprotinin, 100 μg/mL bacitracin, 1 mg/mL bovine serum albumin, 1 mM ATP, 20 mM creatine phosphate, 1 mg/mL phosphocreatine kinase, 10 μM isoproterenol, 10 μM GTP, and various concentrations of peptides (0–10 μM). After the mixture was incubated at 35 °C for 15 min in a shaking water bath, the reaction was arrested by the addition of 100 μM EDTA and boiling for 3 min. cAMP was extracted and quantitated by radioimmunoassay. All the points in the binding and adenylate cyclase studies are the means of three parallel experiments performed in duplicate.

Feeding Studies. Sprague–Dawley rats (350–450 g) with paraventricular hypothalamic cannulae were used to investigate feeding effects of NPY analogs according to our previously published procedures.[5]

Conformational Model Construction. Models of NPY were constructed from the crystal structure of avian pancreatic polypeptide[21] by substituting residues with those from the porcine NPY sequence,[1] using the standard side chain geometry[32] with the Insight/Discover programs (Biosyn Technologies, San Diego, CA). The model of [D-Trp^{32}]NPY was constructed by modifying the dihedral angles of residues 32 and 33 to approximate those

of a standard type II' β-turn, the most probable conformation for this sequence with a D-residue substitution.[32]

Acknowledgment. Supported by grants from the NIH (GM 47122), AICR (91B54), and VA.

References

(1) Tatemoto, K. Neuropeptide Y: complete amino acid sequence of the brain peptide. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5485–5489.
(2) Allen, Y. S.; Adrian, T. E.; Allen, J. M.; Tatemoto, K.; Crow, T. J.; Bloom, S. R.; Polak, J. M. Neuropeptide Y distribution in rat brain. *Science* **1983**, *221*, 877–879.
(3) Stanley, B. G.; Chin, A. S.; Leibowitz, S. F. Neuropeptide Y injected into the paraventricular hypothalamus: a powerful stimulant of feeding behavior. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3940–3943.
(4) Morley, J. E.; Levine, A. S.; Gosnell, B. A.; Kneip, J.; Grace, M. Effect of neuropeptide Y on ingestive behaviors in the rat. *Am. J. Physiol.* **1987**, *252*, R599–R609.
(5) Chance, W. T.; Sheriff, S.; Foley-Nelson, T.; Fischer, J. E.; Balasubramaniam, A. Pertussis toxin inhibits neuropeptide Y induced feeding in rats. *Peptides* **1989**, *10*, 1283–1286.
(6) Kalra, S. P.; Dunbe, M. G.; Sahu, A.; Phelps, C. P.; Kalra, P. S. Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased for food. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10931–10935.
(7) Sahu, A.; Kalra, P.; Kalra, S. P. Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. *Peptides* **1988**, *9*, 83–86.
(8) Sanacora, G.; Kershaw, M.; Finkelstein, J. A.; White, J. D. Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese zucker rats and its regulation by food deprivation. *Endocrinology* **1990**, *127*, 730–737.
(9) Chance, W. T.; Sheriff, S.; Zhang, F.; Kalmonpunpour, M.; Fischer, J. E.; Balasubramaniam, A. Reduction of plasma and hypothalamic neuropeptide Y in anorectic tumor-bearing rats. *Proc. Soc. Neurosci.* **1990**, 774.
(10) Leibowitz, S. F.; Alexander, J. T. Analysis of neuropeptide Y-induced feeding: dissociation of Y_1 and Y_2 receptor effects on natural meal patterns. *Peptides* **1991**, *12*, 1251–1260.
(11) McLaughlin, C. L.; Tou, J. S.; Rogan, G. J.; Baile, C. A. Full amino acid sequence of centrally administered NPY required for maximal food intake response. *Phys. Behav.* **1991**, *49*, 521–526.
(12) Balasubramaniam, A.; Sheriff, S. Neuropeptide Y (18–36) is a competitive antagonist of neuropeptide Y in rat cardiac ventricular membranes. *J. Biol. Chem.* **1990**, *265*, 14724–14727.
(13) Balasubramaniam, A.; Sheriff, S.; Rigel, D. F.; Fischer, J. E. Characterization of neuropeptide Y receptors in rat cardiac ventricular membranes. *Peptides* **1990**, *11*, 545–550.
(14) Michel, M. C. Receptors for neuropeptide Y: multiple subtypes and multiple second messengers. *Trends Pharmacol. Sci.* **1991**, *12*, 389–394.
(15) Shen, G. H.; Grundemar, L.; Zukowska-Grojec, Z.; Hakanson, R.; Wahlestedt, W. C-terminal neuropeptide Y fragments are mast cell-dependent vasodepressor agents. *Eur. J. Pharmacol.* **1991**, *204*, 249–256.
(16) Sheriff, S.; Balasubramaniam, A. Inhibitory and stimulatory effects of neuropeptide Y(17–36): structure-function studies. *J. Biol. Chem.* **1992**, *267*, 4680–4685.
(17) Freidinger, R. M.; Colton, C. D.; Randal, W. C.; Pitzenberger, S. M.; Veber, D. F. A cyclic hexapeptide LH-RH antagonist. *Peptides: Structure and Function*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; 1990; pp 549–552.
(18) Goldman, M. E.; McKee, R. L.; Caulfield, M. P.; Reagan, J. E.; Levy, J. J.; Gay, C. T.; DeHaven, P. A.; Rosenblatt, M.; Chorev, M. A new highly potent parathyroid hormone antagonist: [D-Trp^{12}, Tyr^{34}]bPTH-(7-34)NH_2. *Endocrinology* **1988**, *123*, 2597–2599.
(19) Beck-Sickinger, A.; Gaida, W.; Schnorrenberg, G.; Lang, R.; Jung, G. Neuropeptide Y: Identification of the binding site. *Int. J. Peptide Protein Res.* **1990**, *36*, 522–530.
(20) Tatemoto, K. Neuropeptide Y and its receptor antagonists: use of an analog mixture strategy. *Ann. N.Y. Acad. Sci.* **1990**, *611*, 1–6.
(21) Blundell, T. L.; Pitts, J. E.; Tickle, I. F.; Wood, S. P.; Wu, C. W. X-ray analysis (1.4-Å resolution) of avian pancreatic polypeptide: small globular protein hormone. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4175–4179.
(22) Cowley, D. J.; Hoflack, J. M.; Pelton, J. T.; Saudek, V. Structure of neuropeptide Y dimer in solution. *Eur. J. Biochem.* **1990**, *25*, 1099–1106.
(23) Veber, D. F. Design and discovery in the development of peptide analogs. *Peptides: Chemistry and Biology*; Simith, J. A., Rivier, J. E., Eds.; 1991; pp 2–14.
(24) Sheriff, S.; Balasubramaniam, A.; Krstenansky, J.; Chance, W. T. Tests of agonists effects of NPY analogs. *Proc. Soc. Neurosci.* **1992**, 895.
(25) Kalra, S. P.; Dunbe, M. G.; Fournier, A.; Kalra, P. S. Structure-function analysis of stimulation of food intake by neropeptide Y: effects of receptor agonists. *Physiol. Behav.* **1991**, *50*, 5–9.
(26) Beck, A.; Jung, G.; Gaida, W.; Koppen, H.; Lang, R.; Schnorrenberg, G. Highly potent and small neuropeptide Y agonist obtained by linking NPY 1–4 via a spacer to α-helical NPY 25–36. *FEBS Lett.* **1989**, *1*, 119–122.
(27) Lundberg, J. M.; Hokfelt, T.; Hemsen, A.; Theodorsson-Norheim, E.; Pemow, J.; Hamberger, B.; Goldstein, M. Neuropeptide Y-like immunoreactivity in adrenaline cells of adrenal medulla and in tumors and plasma of pheochromocytoma patients. *Reg. Pept.* **1986**, *13*, 169–182.
(28) Maisel, A. S.; Scott, N. A.; Motulsky, H. J.; Michel, M. C.; Boublik, J. H.; Rivier, J. E.; Ziegler, M.; Allen, R. S.; Brown, M. R. Elevation of plasma neuropeptide Y levels in congestive heart failure. *Am. J. Med.* **1989**, *86*, 43–48.
(29) Balasubramaniam, A.; Grupp, L.; Srivastava, L.; Tatemoto, K.; Murphy, R. F.; Joffe, S. N.; Fischer, J. E. Synthesis of Neuropeptide Y. *Int. J. Peptide Protein Res.* **1987**, *29*, 78–83.
(30) Unden, A.; Tatemoto, K.; Mutt, V.; Bartfai, T. Neuropeptide Y receptor in rat brain. *Eur. J. Biochem.* **1984**, *145*, 525–530.
(31) Westlind-Danielsson, A.; Unden, A.; Abens, J.; Andell, S.; Bartfai, T. Neuropeptide Y receptors and the inhibition of adenylate cyclase in human frontal and temporal cortex. *Neurosci. Lett.* **1987**, *74*, 237–242.
(32) Rose, G. D.; Gierasch, L. M.; Smith, J. A. Turns in Peptides and Proteins. *Adv. Protein Chem.* **1985**, *37*, 1–109.