Nonpeptide Peptidomimetic Antagonists of the Neuropeptide Y Receptor: Benextramine Analogs with Selectivity for the Peripheral Y_2 Receptor

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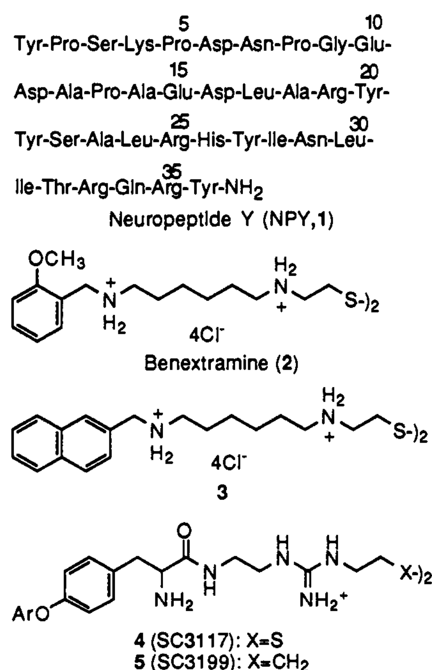
Received February 9, 1994®

We synthesized a new series of benextramine analogs as neuropeptide Y (NPY) functional group mimetics and tested them for *N*-[*propionyl*-³H]NPY ([³]NPY) displacement activity in rat brain membrane homogenates and for NPY receptor antagonist activity in the rat femoral artery. The tetraamine, carbon analog *N,N'*-bis[6-[*N*-(2-naphthylmethyl)amino]hexyl]-1,6-hexanediamine (**15**) was equipotent with benextramine (based on comparison of the relevant IC_{50}'s) in a rat brain [³H]NPY displacement assay, suggesting that the disulfide is not a necessary feature of benextramine's [³H]NPY displacement activity, although this analog maintained selectivity for the benextramine-sensitive binding site population. The bis(*N,N*-dialkylguanyl) disulfide and carbon analogs **14a–c** were 3–4 times more potent than their respective controls in displacing [³H]NPY from rat brain membrane homogenates with IC_{50}'s ranging from 15 to 18 μM and maintained selectivity for the benextramine-sensitive, Y_1 binding site population. However, the activity of the carbon analog *N,N'*-bis[6-[*N*-(2-naphthylmethyl)amino]hexyl]-*N,N'*-(1,6-hexanediyl)diguanidine tetrahydrochloride (**14b**) showed a different profile in a femoral artery vasoconstriction assay; at 1.0 nM, this analog shifted the concentration–effect curve of the Y_2-selective agonist NPY_{13-36} to the right (pA_2 = 9.2; K_d = 0.63 nM) without a significant change in the maximum effect, while even at 1.0 mM it had no effect on the vasoconstrictive activity of the Y_1-selective agonist [Leu³¹,Pro³⁴]NPY. Thus, the bis(*N,N*-dialkylguanidine) analogs of benextramine are selective, competitive antagonists of the postsynaptic NPY receptor in the femoral artery.

Neuropeptide Y (NPY, **1**; Chart 1) is a 36-amino acid peptide first isolated by Tatemoto[1] from porcine brain but since found to be an abundant mammalian neurotransmitter. NPY is stored and released in both the peripheral and central nervous systems and regulates eating and feeding behavior, cardiovascular tone, memory, learning, and hormone release.[2] NPY is a member of the pancreatic polypeptide (PP) family,[3] a peptide group, all of which are 36 amino acids in length, end in a C-terminal tyrosine amide, and are characterized by a propensity to fold into a hairpin structure referred to as the PP-fold. In the case of NPY, the PP-fold brings the N-terminal tyrosine into proximity of the C-terminal amino acids Arg³³, Arg³⁵, and Tyr³⁶, thus forming the active pharmacophore region.[4] NPY receptors consist of three known subtypes as characterized on the basis of the differential activity of peptide agonists.[5,6] The Y_1 receptor has a requirement for essentially the full length of the endogenous peptide and recognizes [Leu³¹,Pro³⁴]NPY (or [Pro³⁴]NPY) as an agonist,[7] the Y_2 receptor binds both NPY and the C-terminal fragment NPY_{13-36} with near equal potency[8] but does not recognize [Leu³¹,Pro³⁴]NPY,[7] and the Y_3 subtype recognizes all three of the above peptide agonists but is insensitive to the NPY homolog polypeptide YY (PYY).[6]

Our laboratories reported the first nonpeptide antagonist of the neuropeptide Y receptor with both in vitro and in vivo activity.[9] Benextramine (**2**), a tetraamine disulfide first designed by Melchiorre as an irreversible adrenergic receptor antagonist,[10] is also equipotent as a NPY receptor antagonist in the rat femoral artery.[11] Benextramine blocks the vasoconstrictive activity of both [Leu³¹,Pro³⁴]-NPY and NPY_{13-36} in vitro with near equal potency, evidence suggesting that benextramine does not discriminate between the peripheral, postsynaptic Y_1 and Y_2 receptors.[12] However, benextramine competitively displaces a maximum of only 61% of specifically bound [³H]-NPY from rat brain membranes.[9,13] Subsequent analysis of benextramine's selectivity relative to [Leu³¹,Pro³⁴]NPY, NPY_{13-36}, and PYY demonstrated that the benextramine-

Chart 1



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® Abstract published in *Advance ACS Abstracts*, June 1, 1994.

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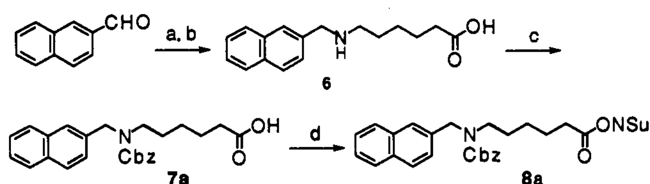
Scheme 1[a]



[a] Reagents: (a) $H_2N(CH_2)_5COOH$/toluene; (b) $NaBH_4/C_2H_5OH$; (c) carbonbenzoxy chloride, $NaHCO_3$; (d) *N*-hydroxysuccinimide, DCC.

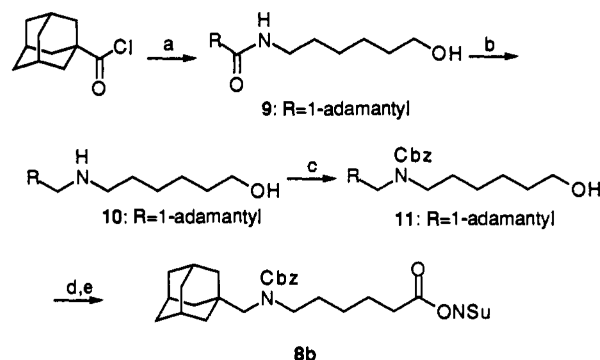
sensitive binding sites in rat brain are of the Y_1 subtype while the benextramine-insensitive sites are of the Y_2 subtype.[13] This evidence first demonstrated a distinct difference between the rat brain Y_2 receptor and the postsynaptic Y_2 receptor in the rat periphery. In addition, Michel and Motulsky[14] reported that He90481 is a nonpeptide antagonist of NPY receptors in vitro.

Although benextramine is not a specific antagonist at NPY receptors, we identified benextramine as a lead useful for the design of more selective analogs. For example, compounds chemically or pharmacologically related to benextramine are without significant activity at the NPY receptor,[9] and we demonstrated through the design and synthesis of benextramine analogs modified in the aromatic ring that the benextramine binding site on NPY receptors is distinctly different from that on adrenergic receptors.[15] Thus, we identified the 2-naphthylmethyl analog **3** as a candidate for further SAR studies in this antagonist series. We also designed a new class of NPY receptor antagonists that contain diaryl and diguanidinium groups designed to mimic the Tyr[1], Tyr[36], Arg[33], and Arg[35] functional groups of NPY. The carbon analog *N,N'*-bis[2-[*N*-[*O*-(2,6-dichlorobenzyl)-L-tyrosyl]amino]ethyl]-*N,N'*-(1,6-hexanediyl)diguanidine (SC3199, **5**) and the disulfide analog [*N,N'*-bis[2-[*N*-[*O*-(2,6-dichlorobenzyl)-L-tyrosyl]amino]ethyl]-guanyl]cystamine (SC3117, **4**) are as potent and 3 times more potent, respectively, than benextramine in displacing [^{3}H]NPY from benextramine-sensitive sites in rat brain but are 8 and 67 times, respectively, mor potent than benextramine as an antagonist at postsynaptic receptors in the femoral artery.[11] In the current study, we modified the benextramine skeleton to test the hypothesis that benextramine is also a function group mimetic of the NPY pharmacophore. We tested the importance of both the disulfide and secondary alkylamine moieties of benextramine on its NPY receptor binding and antagonist activities. In this study, we limited our activity assays to NPY receptors in the rat brain and periphery since the rat is often used as a model for investigating the central and peripheral activities of NPY.

Chemistry

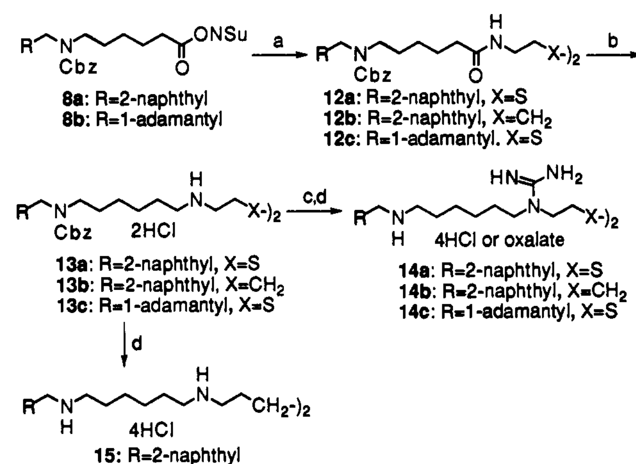
Schemes 1–3 outline our synthetic methods to the bis-(*N,N*-dialkylguanidine) and carbon analogs of benextramine. The common intermediate is the *N*-hydroxysuccinimide-activated ester of 6-(*N*-alkyl-*N*-carbobenzoxyamino)hexanoic acid **8**. Condensation of 2-naphthaldehyde with 6-aminohexanoic acid and subsequent reduction of the imine with sodium borohydride following the method analogous to that of Melchiorre and co-workers[16] yielded 6-[(2-naphthylmethyl)amino]hexanoic acid (**6**). Reaction of acid **6** with carbobenzoxy chloride gave the Cbz-protected amino acid **7a**, and subsequent activation of **7a** with dicyclohexylcarbodiimide and *N*-hydroxysuccinimide at 0 °C gave the active ester **8a**.

Scheme 2[a]



[a] Reagents: (a) $H_2N(CH_2)_6OH$/DIEA; (b) diborane; (c) carbobenzoxy chloride, $NaHCO_3$; (d) CrO_3/H_2SO_4; (e) *N*-hydroxysuccinimide, DCC.

Scheme 3[a]



[a] Reagents: (a) cystamine or hexanediamine, DIEA; (b) diborane; (c) cyanamide, TEA; (d) 30% HBr/HOAc.

The instability of 1-adamantane aldehyde thwarted our initial attempts to synthesize the key adamantyl intermediate **8b** in a manner analogous to that of **8a**. We therefore modified the synthetic sequence as illustrated in Scheme 2. Acylation of 6-aminohexanol with 1-adamantanecarbonyl chloride and subsequent reduction of amide **9** with borane gave the amino alcohol **10**, and reaction with carbobenzoxy chloride gave the *N*-carbobenzoxyamino alcohol **11**. We first attempted to obtain the intermediate disulfide **13c** via conversion of alcohol **11** to the corresponding aldehyde followed by condensation with cystamine and reduction of the imine. However, oxidation of **11** using Swern's reagent[17] or Corey's reagent (pyridinium chlorochromate[18]) did not give a clean product. Therefore, we converted alcohol **11** to the corresponding acid in 70% yield using Jones' reagent,[19] keeping the reaction temperature below 15 °C to minimize concomitant removal of the Cbz protecting group. Treatment of the acid with DCC and *N*-hydroxysuccinimide gave the activated ester **8b**.

Coupling of the ester-activated acid **8a,b** with either cystamine or hexanediamine and subsequent reduction of the amide with borane in refluxing THF[20] gave the corresponding diamines **13a–c**. This sequence now allowed selective guanylation of the unprotected nitrogen. We introduced the guanidinium moieties by reaction of amine **13** with an excess of cyanamide following a modification of a previously reported method.[11] The final step in the

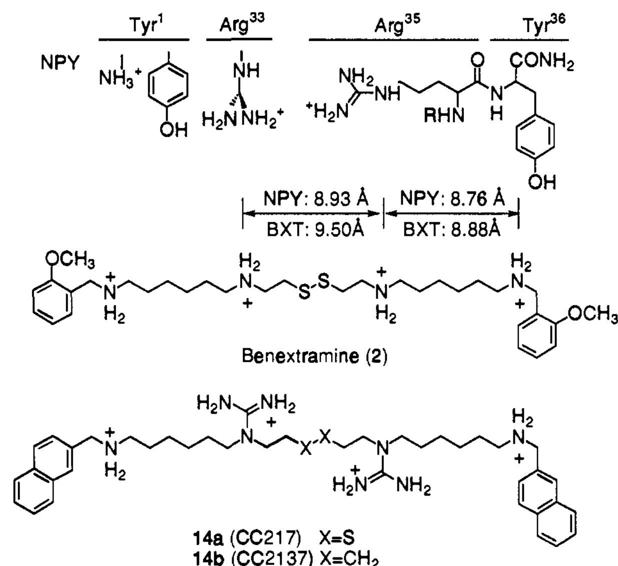


Figure 1. Structural comparisons between a NPY pharmacophore model, benextramine, and NPY functional group mimetics **14a,b**. Measurements (in Å) were made in Sybyl from a PP-fold model of NPY and a linear, minimized model of benextramine.

sequence required removal of the carbobenzoxy protecting group using 30% HBr in acetic acid.

Results and Discussion

We discovered benextramine (**2**) as an NPY receptor antagonist, based on comparison of its chemical composition to the presumed NPY pharmacophore region.[4] In this model (Figure 1), when one benzylic moiety of benextramine overlays with the side chain of Tyr[36], benextramine's aliphatic, secondary amines overlay in an extended structure with the guanidinium groups of Arg[33] and Arg[35]. In addition, benextramine is an irreversible ligand for NPY binding sites in the rat brain[13] and pig spleen[21] and an irreversible antagonist of the Y_1 and Y_2 NPY receptors in the rat femoral artery,[12] apparently due to a thiol–disulfide exchange with a receptor Cys residue. In an extension of this SAR model, we studied the importance of the disulfide and aliphatic, secondary amines of benextramine on its NPY receptor binding and antagonist activity using the NPY versus adrenergic receptor-selective benextramine analog **3** as a lead structure. Figure 1 illustrates our structural comparisons between the NPY pharmacophore region model, benextramine, and the novel NPY receptor antagonists **14a** (CC217) and **14b** (CC2137). We selectively replaced the secondary, aliphatic amines of **3** with *N,N*-dialkylguanidinium groups to approximate more closely the guanidinium groups of Arg[33] and Arg[35] of the NPY pharmacophore region. We refer to these benextramine analogs as NPY functional group mimetics because they mimic the spatial (but not the stereochemical) disposition of the NPY pharmacophore functional groups.

Table 1 lists the activities of these NPY functional group mimetics in displacing specifically bound [^{3}H]NPY from whole rat brain membrane homogenate. As with benextramine,[13] these analogs displace less than 100% of specifically bound [^{3}H]NPY at saturation, so we fit the displacement data to a two-site binding isotherm (using >10 points/curve) with one-site binding affinity as previously described.[22] Nonlinear regression analysis of the binding data gave an IC_{50} for displacement of [^{3}H]NPY

Table 1. Comparison of the [^{3}H]NPY Displacement Activity of NPY Functional Group Mimetics in Rat Brain

R–NH–(CH2)8–X–CH2CH2–Y–)2

compd	R	X	Y	IC_{50} (μM)[a]	B_{max} (%)[b]	RP
2	*o*-CH3OPh	NH	S	57.6 ± 7.1	65	1
3	naphthyl	NH	S	31.3 ± 2.9	68	1.8[c]
15	naphthyl	NH	CH2	48.3 ± 4.0	75	1
14a	naphthyl	N-C(NH2)=NH	S	14.8 ± 2.1	76	3.9[c]
14b	naphthyl	N-C(NH2)=NH	CH2	18.4 ± 3.2	72	2.6[d]
14c	adamantyl	N-C(NH2)=NH	S	15.0 ± 2.1	68	3.8[c]

[a] The IC_{50} (±SD) is the concentration producing half-maximal displacement of [^{3}H]NPY specific binding from benextramine-sensitive NPY binding sites in rat brain membranes. [b] B_{max} (±SD) is the percentage of maximum displacement of 1.0 nM [^{3}H]NPY from rat brain membrane homogenates using 1.0 μM NPY to determine nonspecific binding. [c] Relative potency in displacing 1 nM [^{3}H]NPY from benextramine-sensitive rat brain NPY sites with respect to that of **2**, calculated by dividing the IC_{50} of **2** by the compound's IC_{50}. [d] Relative potency in displacing 1 nM [^{3}H]NPY from benextramine-sensitive rat brain NPY sites with respect to that of **15**, calculated by dividing the IC_{50} of **15** by the compound's IC_{50}.

from the sensitive sites and the percent maximum displacement. We analyzed the analogs' selectivity by modification of the "paired tube" test as previously described;[15] in all cases tested, the compounds showed selectivity for the benextramine-sensitive sites (data not shown). The percent maximum displacement ranged from 65% to 75% in this series, with the variability in maximum displacement apparently being a reflection of the variability in the proportion of the benextramine-sensitive sites. For example, the rat brain cortex contains exclusively the benextramine-sensitive sites since benextramine displaces 100% of specifically bound [^{3}H]NPY from homogenates of this brain region (Doughty and Hu, unpublished observations).

The 2-naphthyl disulfide analog **3** is approximately 2-fold more potent than benextramine at the benextramine-sensitive sites in rat brain.[15] Using the IC_{50} as a measure of activity, the carbon analog **15** is approximately equipotent with benextramine (Table 1), suggesting that the disulfide of benextramine (and of **3**) is not required for NPY displacement activity. Although this result contrasts with benextramine SAR at adrenergic receptors where conversion from the disulfide analog to the carbon analog reduced activity by greater than 100-fold,[23] the activity of this carbon analog at NPY sites in rat brain is consistent with our observation that the carbon analog **5** is only 3-fold less active than the disulfide **4** in rat brain, based on comparison of the relevant IC_{50}'s.[11] However, the IC_{50}'s of benextramine and its disulfide analogs contain a kinetic term for irreversible modification of the NPY receptor.[13] Although we can not quantify the contribution of the covalent modification to benextramine's activity, it should be significant. Thus, the carbon analogs have similar if not greater affinity for the NPY binding sites relative to the disulfides.

Both the disulfide and carbon analogs of the bis(*N,N*-dialkylguanidinium) analogs **14a,b** displayed a significant 3–4-fold increase in [^{3}H]NPY displacement activity in rat brain homogenate relative to **3** and **15**, respectively, although they retained selectivity for the benextramine-sensitive site. As another part of our SAR studies, we also investigated the importance of the 2-naphthyl aromatic rings of **14a** on its displacement activity. We chose the

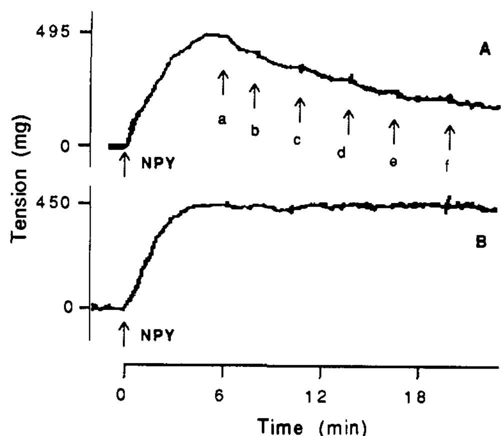


Figure 2. NPY blocking activity of compound **14b** (A) versus that of control (B) in femoral artery rings. The vessel rings were exposed to 5 μM NPY, and once maximum constriction was established, compound **14b** (A) or saline (B) was added every 3 min. Concentrations of **14b**: (a) 10^{-11}; (b) 10^{-10}; (c) 10^{-9}; (d) 10^{-8}; (e) 10^{-7}; and (f) 10^{-6} M.

highly hydrophobic and bulky side chain 1-adamantyl for these studies. The corresponding 1-adamantyl guanidinium disulfide **14c** was equipotent with **14a** in the rat brain [^{3}H]NPY displacement assay and also maintained selectivity at the benextramine-sensitive site. Thus, although the benextramine series removal of the aromatic rings abolishes activity at NPY sites in rat brain,[15] in the bis(*N*,*N*-dialkylguanidine) series, bulky aliphatic groups functionally mimic the 2-naphthylmethyl groups.

The increased activities of the bis(*N*,*N*-dialkylguanidines) **14a–c** relative to those of the corresponding secondary amines in the rat brain [^{3}H]NPY displacement assay correlate with the observed increased activities of the bis(*N*,*N'*-dialkylguanidines) **4** and **5**.[11] Importantly, this latter series showed more potent activities and different selectivities relative to benextramine as NPY receptor antagonists in the rat femoral artery.[11] Thus, we chose the carbon analog **14b** for similar NPY receptor antagonist activity assays in the rat femoral artery. As illustrated by the representative experiment presented in Figure 2, compound **14b** decreased the vasoconstrictive activity of 5 μM NPY in the concentration range between 10^{-11} and 10^{-6} M, an activity about 1000-fold more potent than that of benextramine.[11] However, although benextramine inhibited 100% of the vasoconstrictive activity of NPY,[11] compound **14b** blocked a maximum of only 60.6 ± 4.0% (n = 3) of NPY's activity in this concentration range. Similarly, compounds **4** and **5** only partially block the vasoconstriction activity of 5 μM NPY in the same assay system.[11]

To determine if this absence of complete NPY blocking activity results from a selectivity for the Y_1 versus Y_2 postsynaptic receptors, we analyzed the NPY blocking activity of **14b** versus both the Y_1-selective agonist [Leu^{31},Pro^{34}]NPY and the Y_2-selective agonist NPY_{13-36}; Figure 3 presents the results of these experiments. We observed no significant difference in the vasoconstrictive activity of [Leu^{31},Pro^{34}]NPY in the absence or presence of 1.0 mM **14b** at any tested agonist concentration, evidence suggesting that compound **14b** is not an antagonist of the peripheral, postsynaptic Y_1 receptor up to this concentration. However, 10 nM **14b** shifted the concentration–effect curve of the Y_2-selective agonist NPY_{13-36} by about 2 orders of magnitude, but a combination of both the low solubility and the shallow concentration–effect curve[24] of the peptide agonist prevented us from analyzing the effect of 10 nM **14b** on the maximum vasoconstrictive activity of NPY_{13-36}. However, **14b** at 1.0 nM shifted the concentration–effect curve of NPY_{13-36} from ED_{50} = (6.72 ± 1.9) × 10^{-8} M without antagonist to ED_{50} = (1.80 ± 0.36) × 10^{-7} M in the presence of antagonist, without a corresponding significant decrease in maximum activity (Figure 3). This result suggests that at 1.0 nM the bis(*N*,*N*-dialkylguanidinium) analog **14b** is a reversible, competitive antagonist of the Y_2 postsynaptic NPY receptor with a calculated pA_2 of 9.2.[25]

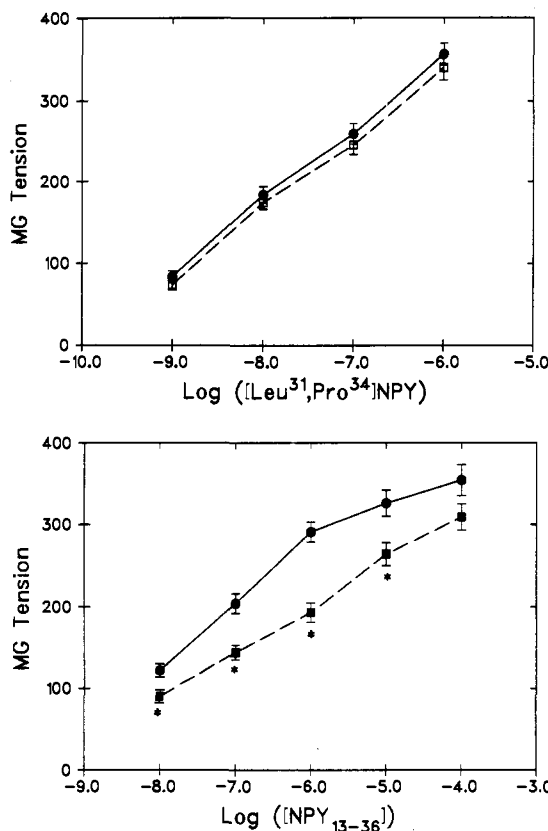


Figure 3. NPY receptor blocking activity of compound **14b** on the agonist activities of [Leu^{31},Pro^{34}]NPY (top) and NPY_{13-36} (bottom) in rat femoral artery rings. The vessel rings were exposed to 1.0 nM (■) or 1.0 mM (□) **14b** for 10 min, and peptide agonist was subsequently applied in log molar increments every 5 min or until maximum constriction was reached, whichever occurred first. Control experiments (●) were treated with saline before the concentration–effect curves. Asterisks indicate significant differences from control values ($p < 0.05$).

In conclusion, the NPY functional group mimetics are weak ligands for the benextramine-sensitive NPY binding sites in rat brain, a site previously characterized as Y_1-like, based on its sensitivity to [Leu^{31},Pro^{34}]NPY,[13] although **14b** shows a much greater activity at the Y_2 postsynaptic receptor in the rat femoral artery. The absence of a direct correlation between rat brain binding activity and peripheral NPY receptor blocking activity provides further evidence for differences in both the Y_1 and Y_2 receptors in the rat brain and the rat periphery. Nevertheless, our current results do correlate with the activity and selectivity of the bis(*N*,*N'*-dialkylguanidine) **5** in the femoral artery versus the rat brain;[11] this analog is also a selective antagonist of the Y_2 postsynaptic receptor in the rat femoral artery.[26] In addition, the disulfide moiety of benextramine is not critical for its activity as a NPY receptor ligand in the rat brain or for postsynaptic, Y_2-

like antagonist activity in the rat femoral artery. This result is additional evidence that the benextramine binding site on the NPY receptors is distinctly different from that on adrenergic receptors.[15]

Experimental Section

Krebs–Ringer bicarbonate buffer, bacitracin, and BSA were purchased from Sigma Chemical Co. (St. Louis, MO), *N*-[*propionyl*-^{3}H]neuropeptide Y ([^{3}H]NPY) was purchased from Amersham (Arlington Heights, IL), and all other solvents and reagents were obtained commercially and used without further purification unless otherwise noted. Melting points were determined on a Thomas-Hoover melting point apparatus and are not corrected. The ^{1}H NMR spectra were recorded on a Bruker AM 500 or QE 300 or a Varian XL 300 spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane as an internal standard. IR spectra were recorded on a Bowmem Fourier transform spectrometer. High-resolution positive-ion fast atom bombardment mass spectra (HRFABMS) were obtained on a VG Analytical ZAB spectrometer with glycerol as matrix. Dr. Nguyen (University of Kansas) performed the microanalyses on a Hewlett-Packard Model 185B CHN analyzer. Methylene chloride was dried by distillation over phosphorous pentoxide. THF was dried by distillation over sodium. Analytical TLC was performed on 0.25-mm silica gel plates (Sigma T6270). Flash column chromatography was carried out with silica gel (Merck, grade 60, 230–400 mesh).

6-[*N*-(2-Naphthylmethyl)amino]hexanoic Acid (6). A mixture of 2-naphthaldehyde (4.1 g, 25.3 mmol) and 6-aminohexanoic acid (3.2 g, 24.5 mmol) in toluene (400 mL) was heated at reflux for 18 h, with water formed during the reaction being removed continuously using a Dean–Stark trap. After the solution cooled to room temperature, the intermediate imine precipitated. The precipitate was collected by filtration, and the filtrate was evaporated under reduced pressure, affording the remainder of the imine as a solid residue. The combined imine intermediate was dissolved in ethanol (700 mL), and sodium borohydride (1.6 g, 42.3 mmol) was added slowly to this solution. After the mixture had stirred for 8 h, the solvent was removed under reduced pressure to give a white solid residue. The residue was dissolved in water (500 mL), and the resulting solution was acidified to pH 2 with 1 N HCl, keeping the solution on an ice–water bath during the addition of the acid. The white precipitate formed was collected by filtration and dried under vacuum. The crude product was recrystallized from methanol–ether to afford 6 g (77%) of **6** as its hydrochloride salt: mp 190–191 °C; IR (KBr) 3655–3250 (COOH, NH), 1705 (CO) cm^{-1}; NMR (DMSO-d_6) δ 12.01 (br s, 1 H, D_2O exchangeable, COOH), 9.38 (br s, 2 H, D_2O exchangeable, NH_2^+), 8.07 (s, 1 H, ArH), 7.97–7.55 (m, 6 H, ArH), 4.20 (s, 2 H, $ArCH_2$), 3.01 (t, J = 7.7 Hz, 2 H, N-CH_2), 2.29 (t, J = 7.2 Hz, 2 H, $COCH_2$), 1.75–1.67 (m, 2 H, CH_2), 1.62–1.53 (m, 2 H, CH_2), 1.41–1.32 (m, 2 H, CH_2). Anal. ($C_{17}H_{21}NO_2HCl$) C, H, N.

6-[*N*-Carbobenzoxy-*N*-(2-naphthylmethyl)amino]hexanoic Acid (7a). Carbobenzoxy chloride (2.8 g, 16.5 mmol) was added dropwise to a mixture of **6** (4.2 g, 13.5 mmol), $NaHCO_3$ (4.8 g, 56.6 mmol), and water (1500 mL) at 0 °C. The reaction mixture was allowed to stir for 6 h, acidified to pH 2 with 1 N HCl, and extracted with ether (3 × 200 mL). The combined ether layer was dried over $MgSO_4$, and the solvent was evaporated under reduced pressure to give a thick pale-yellow oil. Purification of the crude product by flash chromatography (silica gel, 1.5 in. × 10 in., CH_2Cl_2:CH_3OH) afforded 5.4 g (97%) of **7a** as a viscous oil. The oil solidified on standing at room temperature: mp 94–96 °C; IR (KBr) 3655–33545 (COOH), 1712 (COOY), 1659 (CONH) cm^{-1}; NMR (DMSO-d_6) δ 12.01 (br s, 1 H, D_2O exchangeable, COOH), 7.84–7.26 (m, 12 H, ArH), 5.21 (s, 2 H, $ArCH_2$), 4.66 (s, 2 H, $ArCH_2$), 3.32–3.24 (m, 2H, CH_2), 2.31–2.25 (m, 2 H, CH_2), 1.58 (m, 4 H, CH_2), 1.28 (m, 2 H, CH_2). Anal. ($C_{25}H_{24}NO_4$) C, H, N.

6-[*N*-Carbobenzoxy-*N*-(2-naphthylmethyl)amino]hexanoic Acid *N*-Hydroxysuccinimide Ester (8a). A solution of dicyclohexylcarbodiimide (2.7 g, 13.2 mmol) in CH_2Cl_2 (50 mL) was added dropwise to a stirred solution of **7a** (4.9 g, 12.0 mmol) and *N*-hydroxysuccinimide (1.6 g, 13.9 mmol) in CH_2Cl_2 (350 mL) at 0 °C. The solution was allowed to stir for 4 h and then kept in the refrigerator for 10 h. The precipitated solid was removed by filtration, and the filtrate was evaporated at reduced pressure to afford a viscous residue. Purification by flash chromatography (silica gel, 1.5 in. × 10 in., CH_2Cl_2:$CH_3CH_2OCOCH_3$ 9:1) afforded 4.2 g (70%) of the desired product **8a** as a viscous, colorless oil: IR (Nujol) 1812 (CO), 1746 (CO), 1710 (CO) cm^{-1}; NMR ($CDCl_3$) δ 7.85–7.30 (m, 12 H, ArH), 5.22 (d, J = 7.4 Hz, 2 H, $ArCH_2$), 4.68 (s, 2 H, $ArCH_2$), 3.34–3.25 (m, 2H, CH_2), 2.83 (s, 4 H, $COCH_2CH_2CO$), 2.58–2.49 (m, 2 H, CH_2), 1.73–1.29 (m, 6 H, CH_2). Anal. ($C_{29}H_{30}N_2O_6$) C, H, N.

***N*-(6-Hydroxyhexyl)-1-adamantanecarboxamide (9).** A solution of 1-adamantanecarbonyl chloride (0.8 g, 4.1 mmol) in methylene chloride (20 mL) was added dropwise to a stirred solution of 1-aminohexanol (0.5 g, 4.1 mmol) in methylene chloride (60 mL) and triethylamine (1 g, 10.0 mmol) at 5 °C and under a nitrogen atmosphere. The reaction mixture was allowed to stir at room temperature for 18 h and then washed with 0.5 N citric acid (3 × 40 mL). The organic phase was dried (Na_2SO_4), and the solvent was evaporated on a rotary evaporator to give 1.1 g (96%) of a pure white, crystalline powder: mp 98–100 °C; IR (KBr) 3456, 1754 cm^{-1}; NMR ($CDCl_3$) δ 5.72 (br s, 1 H, NH), 3.63 (t, J = 6.42 Hz, 2 H, CH_2), 3.25 (dd, J = 6.90 Hz, 2 H, CH_2), 2.10–1.66 (m, 17 H, adamantyl H, CH_2), 1.45–1.28 (m, 8 H, CH_2).

6-[*N*-(1-Adamantylmethyl)amino]hexanol Hydrochloride (10). A solution of **9** (0.5 g, 1 mmol) in THF (30 mL) was added dropwise to borane–THF (2 mL, 2 mmol) cooled on an ice–water bath and under a N_2 atmosphere. The reaction mixture was heated at reflux for 16 h and then cooled to room temperature. Excess borane was destroyed at 0 °C by careful, dropwise addition of 6 M HCl, and THF was removed by distillation. The pH of the reaction mixture was adjusted to 12 with 1 N NaOH and the mixture was saturated with NaOH pellets (keeping the temperature at 0 °C using an ice–water bath during this addition). The crude amine was extracted in ethyl acetate (3 × 30 mL), the combined organic phase was dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to give an oil. The free base was dissolved in anhydrous ether, and insoluble material was removed by filtration. An ether solution of hydrogen chloride gas was added into the filtrate until precipitation was complete. The precipitated salt was collected by filtration, washed with ether (20 mL), and dried under vacuum to afford 0.34 g (67%) of **10** as a white powder in analytical pure form: mp 171–173 °C; IR (KBr) 3435 cm^{-1}; NMR (D_2O) δ 3.55 (t, J = 6.70 Hz, 2 H, CH_2), 2.99–2.90 (m, 2 H, CH_2), 2.67 (s, 2 H, CH_2), 1.97–1.42 (m, 15 H, adamantyl H), 1.35–1.25 (m, 8 H, CH_2). Anal. ($C_{17}H_{31}N·HCl$) C, H, N.

6-[*N*-Carbobenzoxy-*N*-(1-adamantylmethyl)amino]hexanol (11). This compound was prepared from **10** (4.4 g, 16.5 mmol) following the method described above for the synthesis of **7a**. Purification of the crude product by flash chromatography (silica gel, 1.5 in. × 8 in., CH_2Cl_2:CH_3OH 9.5:5) afforded 5.9 g (84%) of the desired product **11** as a colorless oil: IR (neat) 3422, 1699 cm^{-1}; NMR ($CDCl_3$) δ 7.40–7.28 (m, 5 H, ArH), 5.08 (d, 2 H, ArCH2), 3.57–3.45 (m, 2 H, CH_2), 3.25 (t, J = 6.57 Hz, 2 H, CH_2), 2.95 (s, 2 H, CH_2), 1.98–1.42 (m, 15 H, adamantyl H), 1.40–1.19 (m, 8 H, CH_2). Anal. ($C_{25}H_{37}NO_3$) C, H, N.

6-[*N*-(1-Adamantylmethyl)-*N*-carbobenzoxyamino]hexanoic Acid *N*-Hydroxysuccinimide Ester (8b). Jones' reagent (0.18 mL, 2.67 M) was added in small portions to a solution of **11** (0.12 g, 0.29 mmol) in acetone (3 mL, distilled over $KMnO_4$) cooled on an ice–water bath. After the solution had stirred for 30 min at 10–15 °C, 2-propanol (2 drops) was added to destroy the excess oxidant. The reaction mixture was diluted with water (5 mL), acetone was evaporated on a rotary evaporator, and the aqueous layer was extracted with ether (3 × 15 mL). The combined ether extract was dried over Na_2SO_4, and the solvent was evaporated at reduced pressure to obtain a viscous liquid. Purification by flash chromatography (silica gel, 1 in. × 7 in., CH_2Cl_2:CH_3OH 9:1) afforded 0.08 g (70%) of the acid **7b** as a viscous, colorless oil: IR ($CHCl_3$) 1710, 1695 cm^{-1}; NMR ($CDCl_3$) δ 7.48–7.27 (m, 5 H, ArH), 5.12 (d, 2 H, ArCH2), 3.32–3.18 (m, 2 H, CH_2), 2.95 (s, 2 H, CH_2), 2.40–2.23 (m, 2 H, CH_2), 2.08–1.43 (m, 15 H, adamantyl H), 1.40–1.18 (m, 6 H, CH_2); HRFABMS 414.2614; calcd for $C_{25}H_{35}NO_4$ (M + H)$^+$, 414.2644.

Ester **8b** was obtained from the acid (1.2 g, 2.9 mmol) in 84% yield by the method described above for the synthesis of compound **8a**. **8b**: IR ($CHCl_3$) 1795, 1770, 1715, 1697 cm^{-1}; NMR ($CDCl_3$) δ 7.45–7.30 (m, 5 H, ArH), 5.12 (d, 2 H, ArCH2), 3.35–3.16 (m, 2 H, CH_2), 2.95 (s, 2 H, CH_2), 2.85 (s, 4 H, $-COCH_2CH_2CO-$), 2.65–2.50 (m, 2 H, CH_2), 2.12–1.45 (m, 15 H, adamantyl H), 1.41–1.12 (m, 6 H, CH_2).

***N,N'*-Bis[6-[*N*-carbobenzoxy-*N*-(2-naphthylmethyl)amino]hexanoyl]cystamine (12a).** A solution of **8a** (1.4 g, 2.8 mmol) in CH_2Cl_2 (50 mL) was added to a stirred solution of cystamine (0.2 g, 1.4 mmol) and DIEA (1 mL) in CH_2Cl_2 (100 mL) under a N_2 atmosphere. The reaction mixture was allowed to stir for 18 h, and the solvent was removed under reduced pressure to give a semisolid residue. Excess of DIEA was removed by Kugelrohr distillation (0.05 mmHg, 30 °C), and the crude residue was purified by flash chromatography (silica gel, 1.5 in. × 8 in., CH_2Cl_2:CH_3OH 9:1) to afford 1.0 g of a colorless oil. A second purification (flash chromatography, silica gel, 1 in. × 8 in., ethyl acetate) afforded 0.94 g (72%) of the pure diamide derivative 11 as a thick, colorless, viscous oil: IR (CH_2Cl_2) 3327 (NH), 1682 (CO), 1660 (CONH) cm^{-1}; NMR (DMSO-d_6) δ 7.99–7.22 (m, 28 H, 4 CONH and 24 ArH), 5.06 (d, J = 7.5 Hz, 4 H, $ArCH_2$), 4.56 (s, 4 H, $ArCH_2$), 3.31–3.15 (m, 8H, CH_2, CH_2), 2.71 (t, 4H, CH_2), 1.99 (br s, 4 H, CH_2), 1.42 (br s, 8 H, CH_2), 1.37 (br s, 4 H, CH_2). Anal. ($C_{54}H_{62}N_4O_6{\cdot}S_2{\cdot}0.9H_2O$) C, H, N.

***N,N'*-Bis[6-[*N*-(2-naphthylmethyl)-*N*-carbobenzoxyamino]hexanoyl]-1,6-hexanediamine (12b).** This compound was prepared by the condensation of **8a** (4.0 g, 8.0 mmol) with 1,6-diaminohexane (0.8 g, 4.0 mmol) following the method described for the synthesis of **12a**. Purification of the crude product by flash chromatography afforded 2.8 g (79%) of **12b** as a white solid: mp 105–107 °C; IR (KBr) 3345 (NH), 1679 (CO), 1657 (CONH) cm^{-1}; NMR ($CDCl_3$) δ 7.83–7.27 (m, 24 H, ArH), 5.55 (br s, 2 H, CONH), 5.20 (4 H, $ArCH_2$), 4.64 (s, 4 H, $ArCH_2$), 3.29–3.18 (m, 8 H, CH_2), 2.08–1.99 (m, 4H, CH_2), 1.77–1.21 (m, 20 H, CH_2).

***N,N'*-Bis[6-[*N*-(1-adamantylmethyl)-*N*-carbobenzoxyamino]hexanoyl]cystamine (12c).** This compound was obtained by the condensation of **8b** (1.2 g, 2.4 mmol) with cystamine (0.2 g, 1.2 mmol) following the method described for the preparation of **12a**. Purification of the crude product by flash chromatography (silica gel, 1.5 in. × 7 in., ethyl acetate) afforded 0.6 g (55%) of **12c** as a viscous, colorless oil: IR ($CHCl_3$) 1690, 1656 cm^{-1}; NMR ($CDCl_3$) δ 7.41–7.28 (m, 10 H, ArH), 6.50–6.15 (m, 2 H, NH), 5.10 (d, 4 H, ArCH2), 3.52 (br s, 4 H, CH_2), 3.31–3.20 (m, 4 H, CH_2), 2.94 (s, 4 H, CH_2), 2.80 (t, 4 H, CH_2), 2.25–2.10 (m, 4 H, CH_2), 2.00–1.18 (m, 19 H, adamantyl H, CH_2); HRFABMS 943.5415; calcd for $C_{25}H_{35}NO_4$ $(M + H)^+$, 943.5441.

***N,N'*-Bis[6-[*N*-carbobenzoxy-*N*-(2-naphthylmethyl)amino]hexyl]cystamine Dihydrochloride (13a).** Amide **12a** (0.8 g, 0.8 mmol) was reduced with borane–THF (4 mL, 4 mmol) as described above for the reduction of **10**, yielding 0.5 g of the hydrochloride salt of **13a** as white, hygroscopic powder: IR (KBr) 3659–3282 (NH), 1684 (CO) cm^{-1}; NMR (DMSO-d_6) δ 7.91–7.27 (m, 24, ArH), 5.14 (d, J = Hz, 4 H, $ArCH_2$), 4.60 (s, 4 H, $ArCH_2$), 3.27–3.26 (m, 8 H, CH_2), 3.08–2.85 (m, 8 H, CH_2), 1.56–1.51 (m, 8 H, CH_2), 1.25–1.21 (m, 8 H, CH_2). Anal. ($C_{54}H_{66}N_4O_4{\cdot}S_2{\cdot}1.3H_2O$) C, H, N.

***N,N'*-Bis[6-[*N*-(2-naphthylmethyl)-*N*-carbobenzoxyamino]hexyl]-1,6-hexanediamine Dihydrochloride (13b).** This compound was synthesized by the borane reduction of **12b** (2.3 g, 2.6 mmol) following the method described above for the synthesis of **13a**, yielding 2.0 g (83%) of the hydrochloride salt of **13b** as a white, crystalline solid: mp 130–132 °C; IR (KBr) 3600–3278 (NH), 1680 (CO) cm^{-1}; NMR (CD_3OD) δ 7.87–7.26 (m, 24 H, ArH), 5.20 (4 H, $ArCH_2$), 4.67 (s, 4 H, $ArCH_2$), 3.39–3.28 (m, 8 H, CH_2), 2.93 (m, 4H, CH_2), 1.69–1.25 (m, 24H, CH_2).

***N,N'*-Bis[6-[*N*-(1-adamantylmethyl)-*N*-carbobenzoxyamino]hexyl]cystamine Dihydrochloride (13c).** This compound was prepared in 82% yield by the borane reduction of **12c** following the method described above for the synthesis of **13a**. The free amine was converted to its hydrochloride salt, and the salt was used in the next synthetic step without further purification: mp 142–145 °C; IR (KBr) 3550, 1688 cm^{-1}; NMR (CD_3OD) δ 7.37 (m, 10 H, ArH), 5.10 (d, 4 H, ArCH2), 3.37–3.26 (m, 12 H, CH_2), 3.09 (t, J = 7.41, 7.11 Hz, 4 H, CH_2), 2.97 (s, 4 H, CH_2), 1.98–1.28 (m, 46 H, adamantyl H, CH_2); HRFABMS 915.5854; calcd for $C_{25}H_{35}NO_4$ $(M + H)^+$, 915.5856.

***N,N'*-Bis[6-[*N*-(2-naphthylmethyl)amino]hexyl]-*N,N'*-diguanylcystamine Tetraoxalate (14a).** A solution of **13a** (0.4 g, 0.5 mmol), cyanamide (0.8 g, 19.0 mmol), and triethylamine (0.8 mL) in ethanol (4 mL) was heated at 70 °C for 24 h. The solvent was removed at reduced pressure to give 0.6 g of a light-brown residue. The residue was dissolved in 40 mL of glacial acetic acid and 10 mL of anisole (50 mmol). HBr (16 mL, 30% in glacial acetic acid) was added, and the reaction mixture was allowed to stir at room temperature for 14 h. The precipitated material was removed by filtration, and the filtrate was diluted with anhydrous ether (400 mL). The resulting precipitate was allowed to settle in the refrigerator for 10 h, solvent was decanted, and the solid was dissolved in water (50 mL). The aqueous solution was washed with ether (30 mL), treated with 1 N NaOH to pH 13 to precipitate the free base, and extracted with ethyl acetate (3 × 150 mL). The combined organic phase was dried over $MgSO_4$, and the solvent was evaporated at reduced pressure to give a viscous residue. The crude residue was dissolved in absolute ethanol (50 mL) and filtered to remove insoluble material. The filtrate was treated with a saturated ether solution of anhydrous oxalic acid (50 mL) and allowed to stir for 1 h. The solvent was evaporated under reduced pressure, and the residue was washed with anhydrous ether (2 × 30 mL) to remove traces of oxalic acid. The crude oxalate salt was recrystallized from ethanol–ether to give 0.2 g (37%) of the desired product as a white powder: mp 145–148 °C; IR (KBr) 3342 (NH), 1652 cm^{-1}; NMR (DMSO-d_6) δ 8.15–7.55 (m, 14, ArH), 4.31 (s, 4 H, $ArCH_2$), 3.58–3.51 (m, 4 H, CH_2), 3.27–2.29 (m, 4 H, CH_2), 3.12–2.81 (m, 8 H, CH_2), 1.56–1.51 (m, 16 H, CH_2); HRFABMS 715.4276, calcd for $C_{25}H_{35}NO_4$ $(M + H)^+$, 715.4304.

***N,N'*-Bis[6-[*N*-(2-naphthylmethyl)amino]hexyl]-*N,N'*-(1,6-hexanediyl)diguanidine Tetrahydrochloride (14b).** This compound was synthesized by reaction of **13b** (0.4 g, 0.4 mmol) with an excess of cyanamide and subsequent removal of the carbobenzoxy group following the method described above for the synthesis of **14a**. The free amine obtained from the Cbz deprotection was converted to the hydrochloride salt; recrystallization of the crude hydrochloride salt from ethanol–ether gave 0.1 g (34%) of **14b** as a white solid: mp 212–214 °C; IR (KBr) 3600–3278 (NH), 1680 (CO) cm^{-1}; NMR (D_2O) δ 8.10–8.03 (m, 8 H, ArH), 7.71–7.61 (m, 6 H, ArH), 4.46 (s, 4H, $ArCH_2$), 3.15 (t, J = 7.77 Hz, 4H, CH_2), 3.67–3.05 (dd, J = 6.36, 6.15 Hz, 8 H, CH_2), 1.77–1.68 (m, 12 H, CH_2), 1.51–1.39 (m, 12 H, CH_2); HRFABMS $m/e + 1$ calcd for $C_{42}H_{63}N_8$, 679.5176; found, 679.5194.

***N,N'*-Bis[6-[*N*-(1-adamantylmethyl)amino]hexyl]-*N,N'*-diguanylcystamine Tetrahydrochloride (14c).** This compound was prepared from **13c** (0.8 g, 0.9 mmol) by the method described above for the synthesis of **14a**. The resulting oil was dissolved in ethanol (8 mL), and the hydrochloride salt was prepared by the dropwise addition of an ether solution of hydrogen chloride. The precipitated salt was collected by filtration and recrystallized from ethanol–ether to obtain 0.25 g (33%) of **14c** as a white amorphous solid: mp 206–208 °C; IR (KBr) 3357, 1654 cm^{-1}; NMR (CD_3OD) δ 3.76–3.68 (m, 4 H, CH_2), 3.48–3.40 (m, 4 H, CH_2), 3.10–2.99 (m, 8 H, CH_2), 2.75 (s, 4 H, CH_2), 2.08–1.42 (m, 46 H, adamantyl H, CH_2); HRFABMS 731.5540; calcd for $C_{25}H_{35}NO_4$ $(M + H)^+$, 731.5556.

***N,N'*-Bis[6-[*N*-(2-naphthylmethyl)amino]hexyl]-1,6-hexanediamine Tetrahydrochloride (15).** HBr (30% in acetic acid, 20 mL) was added to a solution of **13a** (0.4 g, 0.4 mmol) in glacial acetic acid (5 mL). The solution was allowed to stir for 14 h, and the precipitate was removed by filtration. HBr and glacial acetic acid were removed by roto-evaporation with methanol to afford a viscous residue. The residue was dissolved in water (50 mL) and washed with ether (3 × 40 mL). The pH of the aqueous layer was adjusted to 13 with 1 N NaOH, and the solution was saturated with NaCl and extracted with ethyl acetate (3 × 50 mL). The combined organic phase was dried over $MgSO_4$, and the solvent was evaporated at reduced pressure to give a viscous residue. The crude product was dissolved in absolute ethanol (10 mL) and filtered. The filtrate was treated with a saturated ether solution of hydrogen chloride. The precipitated salt was collected by filtration, washed with ether, and recrystallized from ethanol–ether to give 0.1 g (32%) of the desired

product: mp 318–320 °C; IR (KBr) 3477 cm^{-1}. NMR (CD_3OD) δ 8.10–7.90 (m, 8 H, ArH), 7.65–7.58 (m, 6 H, ArH), 4.40 (s, 4H, $ArCH_2O$, 3.18–2.92 (m, 12 H, CH_2), 1.85–1.635 (m, 24 H, CH_2); HRFABMS 595.4740; calcd for $C_{25}H_{35}NO_4$ $(M + H)^+$, 595.4737.

Radioligand Binding Assays. Rat brain membranes from male Sprague–Dawley (SD) rats (250–300 g) were prepared by a previously reported method.[13] Membrane homogenate (1.2–1.6 mg of protein/mL), 1.0 nM [^{3}H]NPY, and 10–12 concentrations of 10^{-3}–10^{-7} M compound were incubated in duplicate in assay buffer (Krebs–Ringer bicarbonate buffer containing 50 mM phosphate, 0.1% BSA, 0.05% bicitracin, pH 7.4; 500 μL total volume) at 25 °C for 90 min in siliconized poly(ethylene) culture tubes; controls were incubated similarly with [^{3}H]NPY in the absence (total binding) and presence (nonspecific binding) of 1 μM NPY. After incubation, the membranes were collected by filtration of the assay mixture through siliconized, poly(ethyleneimine)-pretreated Whatman GF/A glass microfiber filters, and the filters were washed with 3 × 3 mL of assay buffer. The filters were dried at 55 °C for 90 min, and the radioactivity remaining on the filters was quantified by scintillation counting in 5 mL of Scintiverse II (Fisher) using a Packard 1900 TR scintillation spectrometer. Each experiment was repeated in at least triplicate, and the IC_{50} values were calculated by fitting the average concentration-dependent [^{3}H]NPY displacement data to a two-site competitive binding isotherm with single-site affinity using the program MINSQ (Micromath, Salt Lake City, UT) as described previously.[22]

Rat Femoral Artery Activity Assays. Assays were conducted according to the procedures of Tessel and co-workers.[27] Briefly, after the induction of unconsciousness with carbon dioxide, SD rats (300–400 g) were decapitated. A 1-cm portion of the right femoral artery proximal to the peritoneal wall was then excised and placed in ice-cold modified Krebs buffer. The arteries were carefully cleaned of extraneous tissue, and the central 3-mm portion of the ring was then mounted on two stainless steel, L-shaped wires while in ice-cold buffer. The mounted rings were suspended in a water-jacketed organ bath containing 3 mL of buffer at 37 °C, maintained at pH 7.4 by gassing with 95% O_2–5% CO_2, and connected at one end to a glass rod and at the other end to a micromanipulator-mounted Grass T3 force transducer (connected to a Grass 7D polygraph) with a 1000-mg resting tension (time 0). This level of resting tension was maintained during the remainder of the experiment. The rings were subsequently exposed twice to 10 μM norepinephrine (NE; 50 μL; once at 30 min and once at 60 min after time 0) until maximal NE-induced constriction occurred. Only rings in which the second NE exposure resulted in at least a 500-mg increase in tension (95% of the vessels evaluated) were used further. In addition, the rings were washed with fresh buffer every 10 min before and after each norepinephrine dose. At time 90 min, 5 μM NPY was applied, and after maximal constriction was established, cumulative doses (from 10^{-11} to 10^{-7}) of antagonist were applied every 3 min. Alternatively, the vessels were incubated with 1 nM or 100 μM antagonist for 10 min before the addition of peptide agonist ([Leu^{31},Pro^{34}]NPY or NPY_{13-36}), and agonist was then applied in log increments after either increases in tension associated with the preceding concentration had reached a new steady level or 5 min had passed, whichever occurred first. For the latter experiments, data were analyzed using analyses of variance in the presence or absence of nonpeptide antagonist as within- and between-subject factors.

Acknowledgment. The authors gratefully acknowledge the American Heart Association, National Center and Kansas Affiliate, and the Inez Jay Research Fund (University of Kansas) for financial support of this research and Dr. Todd Williams, KU Mass Spec Laboratory, for the mass spectra.

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