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Identification of Potent and Selective Neuropeptide Y Y_1 Receptor Agonists with Orexigenic Activity in Vivo

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ABSTRACT

Neuropeptide Y (NPY) binds to a family of G-protein coupled receptors termed Y_1, Y_2, Y_3, Y_4, Y_5, and y_6. The use of various receptor subtype-selective agonists and antagonists has facilitated identification of the receptor subtypes responsible for mediating many of the biological effects of NPY. For example, the potent orexigenic activity of NPY is believed to be mediated by both the Y_1 and Y_5 receptor subtypes. Several selective Y_5 receptor agonists that stimulate food intake in rodents are available, but no selective Y_1 receptor agonist has been reported. We have identified several NPY analogs that bind the NPY Y_1 receptor with high affinity and exhibit full agonist activity, measured as inhibition of forskolin-stimulated cAMP production in cells expressing the cloned NPY Y_1 receptor. [D-Arg^{25}]-NPY, [D-His^{26}]-NPY, Des-AA$^{10-17}$[Cys^{7,21},Pro^{34}]-NPY, Des-AA$^{11-18}$[Cys^{7,21},D-Lys^{9}(Ac)]-NPY, Des-AA$^{11-18}$[Cys^{7,21},D-Lys^{9}(Ac),Pro^{34}]-NPY, Des-AA$^{11-18}$[Cys^{7,21},D-Lys^{9}(Ac),D-His^{26}]-NPY and Des-AA$^{11-18}$[Cys^{7,21},D-Lys^{9}(Ac),D-His^{26},Pro^{34}]-NPY bind the NPY Y_1 receptor with K_i values of 0.9 ± 0.2, 2.0 ± 0.3, 0.2 ± 0.05, 0.7 ± 0.1, 0.2 ± 0.01, 2.2 ± 0.3, and 1.2 ± 0.3 nM, respectively, and inhibit forskolin-stimulated cAMP production with EC_{50} values of 0.2 ± 0.02, 0.5 ± 0.04, 0.3 ± 0.03, 0.5 ± 0.05, 0.4 ± 0.16, 5.3 ± 0.32, and 5.1 ± 0.97 nM, respectively. These peptides are highly selective for the NPY Y_1 receptor relative to the NPY Y_2, Y_4, and Y_5 receptors. [D-Arg^{25}]-NPY, [D-His^{26}]-NPY and Des-AA$^{11-18}$[Cys^{7,21}, D-Lys^{9}(Ac),D-His^{26},Pro^{34}]-NPY stimulate food intake dose-responsively in Long-Evans rats for at least 4 h after intracerebroventricular administration. Although the involvement of Y_1 receptors in several physiological activities, such as vasoconstriction and anxiolysis, remains to be investigated, adequate tools are now available.

NPY, a 36-amino-acid amidated peptide, is a member of the pancreatic polypeptide (PP) family, which also includes PP and the intestinal peptide, peptide YY (PYY). NPY is abundantly expressed in neurons and regulates a variety of physiological activities, including food intake (Heinrichs et al., 1998; Schwartz et al., 2000), energy expenditure (Hwa et al., 1999), anxiolysis (Heilig et al., 1989), vasoconstriction (Lundberg et al., 1982), ethanol consumption (Thiele et al., 1998), learning and memory (Wettstein et al., 1995; Redrobe et al., 1999), nociception (Broqua et al., 1996; Bannon et al., 2000) and anticonvulsant activity (Baraban, 1998; Vezzani et al., 1999). A family of six G-protein coupled receptors, termed Y_1, Y_2, Y_3, Y_4, Y_5, and y_6, has been identified that binds NPY, PYY and/or PP with high affinity. The NPY Y_3 receptor has not yet been cloned, and the *NPY y6 receptor* gene is a pseudogene in primates and is not present in rat (Burkhoff et al., 1998). Unlike NPY, which is expressed ubiquitously in the nervous system, the receptor subtypes show more restricted tissue-specific expression patterns.

NPY is one of a complex network of regulatory molecules produced both centrally and peripherally that act in the hypothalamus to regulate body weight (Williams et al., 2000). Injection of NPY into the cerebral ventricles (Clark et al., 1984) or the paraventricular nucleus (PVN) of the hypothalamus (Stanley and Leibowitz, 1984, 1985) elicits a robust feeding response and reduces body temperature in rodents (Bouali et al., 1995; Currie and Coscina, 1995; Pedrazzini et al., 1998). Chronic administration results in obesity (Vettor et al., 1994). Evidence that endogenous NPY may regulate feeding comes from studies demonstrating that food deprivation increases the content and release of NPY in the PVN and that refeeding reverses these effects (Sahu et al., 1988; Brady et al., 1990; Kalra et al., 1991). Furthermore, leptin, the adipocyte-derived satiety factor, is believed to act as a molecular signal informing NPY-ergic neurons in the PVN of the status of peripheral energy stores. High leptin levels resulting from increased adiposity suppress NPY production, whereas low leptin levels are permissive for hypothalamic NPY production (Campfield et al., 1996). The obesity syndrome of leptin-deficient ob/ob mice is partially attenuated in animals that are deficient in both leptin and NPY (Erickson et al., 1996).

ABBREVIATIONS: NPY, neuropeptide Y; PP, pancreatic polypeptide; PYY, peptide YY; PVN, paraventricular nucleus; CHO, Chinese hamster ovary; HEK, human embryonic kidney; HBSS, Hanks' balanced salt solution.

Pharmacological and genetic approaches have been used to identify the NPY receptor subtypes that mediate feeding. The ability of various NPY analogs to stimulate food intake in rodents generally matches their in vitro affinities for the Y_1 and Y_5 receptor subtypes (Gerald et al., 1996; Hu et al., 1996). Additionally, Y_1- and Y_5-selective receptor antagonists have been shown to reduce spontaneous or NPY-stimulated food intake in rodents (Rudolf et al., 1994; Doods et al., 1995; Kanatani et al., 1996; Matthews et al., 1997; Criscione et al., 1998; Ishihara et al., 1998; Kask et al., 1998; Wieland et al., 1998). In vivo experiments in which expression of the Y_1 or Y_5 receptor subtypes has been blocked by antisense DNA (Lopez-Valpuesta et al., 1996; Tang-Christensen et al., 1998) or by targeted disruption of these genes (Marsh et al., 1998; Pedrazzini et al., 1998; Kanatani et al., 2000) have confirmed the involvement of the Y_1 and Y_5 receptor subtypes in feeding behavior. Within the hypothalamus, a region known to be involved in central regulation of feeding, the NPY Y_1 and/or Y_5 receptor subtypes are expressed in the paraventricular, medial preoptic, supraoptic, and arcuate nuclei and in the lateral hypothalamus (Gerald et al., 1996; Durkin et al., 2000). These areas are near the third ventricle; hence, the role of the Y_1 and Y_5 receptors in feeding can be probed by i.c.v. injection of peptide and nonpeptide agonists and antagonists.

Although selective Y_5 receptor agonists have been identified (Cabrele et al., 2000; Parker et al., 2000), the lack of Y_1-selective agonists has hampered the study of this receptor's role in feeding and obesity. In earlier studies several NPY analogs containing single-point D-amino acid substitutions were found to have different binding affinities for the NPY Y_1 and Y_2 receptor subtypes (Kirby et al., 1993a, 1995). Kirby et al. (1995) also identified a truncated NPY analog, Des-AA$^{10-17}$[Cys^{7,21},Pro^{34}]-NPY, that is selective for the NPY Y_1 receptor over the Y_2 receptor. To determine whether any of these peptides might bind preferentially to the Y_1 receptor relative to the other NPY receptor subtypes, their affinities for all NPY receptors were measured. We report here the identification of several potent and selective NPY Y_1 receptor agonists and their effect on food intake in rats.

Materials and Methods

Peptide Synthesis and Characterization. Peptides were assembled by solid phase peptide synthesis techniques using the tertiary-butyloxycarbonyl strategy. Peptides were purified by preparative high-performance liquid chromatography and characterized using capillary zone electrophoresis, mass spectrometry, and analytical high-performance liquid chromatography as described previously (Kirby et al., 1993b).

Cloning and Expression of the NPY Receptors. Cloning and expression of the human and rat NPY Y_1, Y_2, Y_4, and Y_5 receptors and the construction of a chimeric rat/human NPY Y_5 receptor were described previously (Parker et al., 1998).

Cell Culture. Cell lines expressing one of the NPY receptor subtypes were grown in F12 medium (CHO-K1 cells) or Dulbecco's modified Eagle's medium (HEK 293 cells) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 200 μg/ml G418 or Zeocin.

Competition Binding Assay. Cell membranes were prepared as described by Parker et al. (1998). Membrane protein (5–10 μg) was incubated with 0.2 nM ^{125}I-porcine PYY (NPY Y_1, Y_2, and Y_5 receptors) or ^{125}I-human PP (NPY Y_4 receptor) and the nonradiolabeled peptide of interest (10^{-11}-10^{-6} M) in 50 mM HEPES, pH 7.2, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.1% (w/v) bovine serum albumin in a total volume of 200 μl for 90 min at room temperature (NPY Y_2, Y_4, and Y_5 receptors) or at 30°C (NPY Y_1 receptor). Nonspecific binding was determined using 10^{-6} M human/rat NPY (NPY Y_1, Y_2, and Y_5 receptors) or human PP (NPY Y_4 receptor). The reaction mixtures were filtered through Millipore MAFC glass fiber filter plates presoaked in 0.5% (v/v) polyethylenimine. The filters were washed twice with 150 μl of ice-cold Dulbecco's phosphate-buffered saline, and the filter-bound radioactivity was measured in a Packard TopCount scintillation counter (Packard, Meriden, CT).

cAMP Assay. HEK 293 cells expressing the human NPY Y_1 receptor, the rat NPY Y_4 receptor, or the rat NPY Y_5 receptor or CHO cells expressing the human NPY Y_2 receptor were used to measure cAMP production. Cells were plated at 1.5×10^4 cells/well in 96-well dishes and reached confluence in 3 days. The cell monolayers were washed once in Hanks' balanced salt solution (HBSS), then incubated for 20 min at 37°C in HBSS containing 10 mM HEPES, pH 7.2, 4 mM $MgCl_2$, 0.2% (w/v) bovine serum albumin, and 1 mM 3-isobutyl-1-methylxanthine. This solution was then replaced with HBSS containing 10 mM HEPES, pH 7.2, 4 mM $MgCl_2$, 0.2% (w/v) bovine serum albumin, 1 mM 3-isobutyl-1-methylxanthine, forskolin (HEK 293 cells, 2.5 μM; CHO cells; 5 μM) and NPY or an NPY analog (10^{-15}-10^{-4} M). The incubation was allowed to continue for an additional 10 min. Intracellular cAMP was then extracted with 75 μl of 100% ethanol and quantified by radioimmunoassay (Flash Plate; PerkinElmer, Boston, MA).

Animals. Adult male Long-Evans rats (250–300 g) were maintained in individual cages at 22°C with free access to food (Teklad rodent diet 8604; Harlan, Madison, WI) and water. The rats were exposed to a 12 h/12 h light/dark cycle with lights on at 4:00 AM. All studies were conducted in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care following protocols approved by the Animal Care and Use Committee of Schering-Plough Research Institute. The procedures were performed in accordance with the principles and guidelines established by the National Institutes of Health for the care and use of laboratory animals.

Surgery. Long-Evans rats were anesthetized by intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). A 22-gauge stainless steel cannula was implanted into the lateral ventricle using the following stereotaxic coordinates for cannula placement: 1.0 mm posterior to bregma, 1.5 mm lateral to midline, and 3.6 mm ventral to dura. Three weeks after surgery, the correct placement of the cannula was ascertained by measuring food intake after i.c.v. administration of NPY (0.3 nmol). Rats consuming at least 2.0 g of food within 60 min of NPY administration were judged to have correctly implanted cannulas.

Intracerebroventricular Infusion Protocols. All peptides administered to rats were dissolved in 0.9% sterile NaCl and administered at doses of 0.1, 0.3, or 1.0 nmol. Intracerebroventricular infusions were given in the middle of the light cycle (i.e., at 10:00 AM) to satiated rats. Peptides were infused into the lateral ventricle at 5 μl/min using a Hamilton infusion pump and syringe (Bioanalytical Systems, West Lafayette, IN). The guide cannula remained inserted for 1 min after infusion to prevent backflow of the infusion solution up the needle track. A known amount of chow was made available to the rats immediately after peptide infusion. Food consumption was measured by subtracting the amount of chow remaining 1, 2, or 4 h after peptide infusion from the amount presented at the beginning of the experiment. Baseline food consumption was determined 3 days before peptide administration by measuring food intake 1, 2, and 4 h after i.c.v. infusion of 0.9% NaCl.

Data Analysis. Binding and cAMP data were analyzed by nonlinear regression analysis using Prism (Graph Pad, San Diego, CA). In vivo results are given as means ± S.E. For each Y_1 agonist, changes in food intake were analyzed using analysis of variance followed by Dunnett's multiple comparisons test. Statistical signifi-

cance was assessed by the $p < 0.05$ between the effect of the saline control and each dose of agonist or between the agonist alone and the agonist plus an antagonist.

Results

Two Y_1-selective NPY analogs were identified from the complete series of D-amino acid substituted peptides, [D-Arg^{25}]-NPY (**1**) and [D-His^{26}]-NPY (**2**). **1** binds the Y_1 receptor with 12-, 82-, and 48-fold higher affinity than the Y_2, Y_4, and Y_5 receptor subtypes, respectively. Similarly, the affinity of **2** for the Y_1 receptor is 14-, 10-, and 17-fold greater than for the Y_2, Y_4, and Y_5 receptor subtypes, respectively. The affinity of **1** for the NPY Y_1 receptor is 3.2 times less than that of NPY, whereas the affinity of **2** is 7.1 times less than that of NPY (Table 1). Substitution of both residues{[D-Arg^{25},D-His^{26}]-NPY (**3**)} yielded a molecule with a higher degree of selectivity for the NPY Y_1 receptor over the Y_2, Y_4, and Y_5 receptors but with a significantly higher Y_1 K_i value (9.7 nM) than either **1** or **2** (Table 1).

A second set of cyclized NPY analogs lacking the internal residues that make up the putative β turn were also evaluated for receptor subtype selectivity. Des-AA$^{10-17}$[Cys^{7,21},Pro^{34}]-NPY (**4**) binds the NPY Y_1 receptor with a K_i value of 0.2 nM, whereas Des-AA$^{11-18}$[Cys^{7,21},D-Lys^{9}(Ac)]-NPY (**5**) has a K_i value of 0.7 nM. Both peptides are greater than 100-fold selective for the NPY Y_1 over the NPY Y_5 receptor (Table 1). However, **4** binds the NPY Y_4 receptor with relatively high affinity (5.0 nM), and **5** binds the NPY Y_2 receptor with a K_i value of 1.4 nM. Des-AA$^{11-18}$[Cys^{7,21},D-Lys^{9}(Ac),Pro^{34}]-NPY (**6**) has lower affinity for the Y_2 receptor (K_i = 124 nM) and higher affinity for the Y_1 receptor (K_i = 0.2 nM) but also higher affinity for the NPY Y_4 receptor (K_i = 7.3 nM). Substitution of D-His at position 26 {Des-AA$^{11-18}$[Cys^{7,21},D-Lys^{9}(Ac), D-His^{26}]-NPY (**7**)} results in marked loss of affinity for the NPY Y_1 (K_i = 2.2 nM), NPY Y_4 (K_i = 206 nM), and NPY Y_5 (K_i = 3023 nM) receptors but an increased affinity for the NPY Y_2 receptor (1.4 nM), and thus a loss of Y_1/Y_2 selectivity. However, substitution of both D-His^{26} and Pro^{34} {Des-AA$^{11-18}$[Cys^{7,21},D-Lys^{9}(Ac),D-His^{26},Pro^{34}]-NPY (**8**)} yields a peptide that is 645-fold selective for the NPY Y_1 receptor over the NPY Y_2 receptor, 26-fold selective for the NPY Y_1 receptor over the NPY Y_4 receptor, and 1900-fold selective for the NPY Y_1 receptor over the NPY Y_5 receptor, although it retains a K_i value of 1.2 nM for the Y_1 receptor.

The functional activity of these NPY Y_1-selective peptides was determined by measuring their ability to block forskolin-stimulated cAMP production in cell lines expressing one of the NPY receptor subtypes. The results are shown in Table 2 and Fig. 1. The EC_{50} value of NPY for the human NPY Y_1 receptor expressed in HEK 293 cells is 0.6 nM. All of the NPY Y_1-selective peptides tested are full agonists for the NPY Y_1 receptor, exhibiting varying degrees of potency ranging from an EC_{50} value of 0.2 nM for **1** to 13.6 nM for **3**. **1**, **2**, **7**, and **8** are highly selectivity for the NPY Y_1 receptor subtype, whereas **4**, **5**, and **6** are also potent agonists for either the NPY Y_2 or NPY Y_4 receptors.

Four of the peptides identified as selective NPY Y_1 receptor agonists in vitro, **1**, **2**, **4**, and **8**, were compared with NPY for their ability to stimulate food intake in satiated Long-Evans rats. The peptides were administered at doses of 0.1, 0.3, or 1.0 nmol, and food consumption was monitored over a 4-h period (**1**, **2**, and **8**) or a 1-h period (**4**). Three of the agonists tested (**1**, **2**, and **8**) stimulated food intake in a dose- and time-dependent manner (Fig. 2, a-c). The increase in food consumption observed after administration of the three active NPY Y_1 agonists or NPY was apparent within 1 h and was sustained for at least 4 h. Relative to the saline control group, NPY and **2** significantly stimulated food intake 1, 2, and 4 h after dosing at doses of 0.3 and 1.0 nmol, whereas **1** significantly stimulated food intake at a dose of 1 nmol 1 and 2 h after administration and at the 0.3 nmol and 1 nmol doses 4 h after administration. The stimulation of food intake elicited by **8** was not statistically significant relative to the saline control but did show a significant linear ($p < 0.05$) overall dose-dependent trend at each time point. **4** did not stimulate food intake after 1 h at the 1.0 nmol dose relative to the saline control (data not shown).

The dimeric nonapeptide 1229U91 (also referred to as GR231118 and GW1229) is a potent and selective antagonist of the NPY Y_1 receptor (Daniels et al., 1995) and has been reported to inhibit NPY-stimulated food intake in rats (Kanatani et al., 1996; Ishihara et al., 1998; Widdowson et al., 1999). We examined the ability of 1229U91 to block feeding stimulated by **1** (Fig. 3). Two-hour food intake was stimulated 2.7-fold by **1** (0.3 nmol). Coadministration of **1** (0.3 nmol) and 1229U91 (3 nmol) completely inhibited **1**-stimulated food intake.

TABLE 1

Affinities of NPY, PP and NPY analogs for the cloned human NPY Y_1, Y_2, Y_4, and Y_5 receptor subtypes.

Competition binding assays were performed using membranes prepared from CHO-K1 cells expressing one of the human NPY receptor subtypes. Binding assays were performed in duplicate. Each value is the mean ± S.E.M. of three to four determinations.

Peptide	K_i			
	Y_1	Y_2	Y_4	Y_5
	nM			
NPY	0.28 ± 0.06	1.2 ± 0.3	N.D.	1.5 ± 0.1
PP	N.D.	N.D.	0.07 ± 0.03	N.D.
1 [D-Arg^{25}]-NPY	0.9 ± 0.2	11.6 ± 2.9	74.6 ± 21.3	43.4 ± 4.2
2 [D-His^{26}]-NPY	2.0 ± 0.3	29.0 ± 4.7	20.1 ± 1.9	34.6 ± 3.8
3 [D-Arg^{25}, D-His^{26}]-NPY	9.7 ± 0.6	399 ± 35.5	852 ± 114	3003 ± 440
4 Des-AA$^{10-17}$ [Cys^{7,21}, Pro^{34}]-NPY	0.2 ± 0.05	37.0 ± 12.4	5.0 ± 1.8	22.6 ± 5.0
5 Des-AA$^{11-18}$ [Cys^{7,21}, D-Lys^{9} (Ac)]-NPY	0.7 ± 0.1	1.4 ± 0.1	53.5 ± 7.9	77.1 ± 36.1
6 Des-AA$^{11-18}$ [Cys^{7,21}, D-Lys^{9} (Ac), Pro^{34}]-NPY	0.2 ± 0.01	124 ± 11.3	7.3 ± 1.1	55.9 ± 7.4
7 Des-AA$^{11-18}$ [Cys^{7,21}, D-Lys^{9} (Ac), D-His^{26}]-NPY	2.2 ± 0.3	1.4 ± 0.3	206 ± 16.8	3023 ± 164
8 Des-AA$^{11-18}$ [Cys^{7,21}, D-Lys^{9} (Ac), D-His^{26}, Pro^{34}]-NPY	1.2 ± 0.1	801 ± 41.4	31.4 ± 1.3	2363 ± 142

N.D., not determined.

Discussion

The study of the physiological responses mediated by each NPY receptor subtype has been facilitated by the identification of subtype-selective agonists. Agonists selective for the Y_2 receptor [C2-NPY (McLean et al., 1990)], the Y_4 receptor [rat PP (Bard et al., 1995)], and the Y_5 receptor {[DTrp^{32}]NPY (Balasubramaniam et al., 1994), [DTrp^{34}]NPY (Parker et al., 2000), [Ala^{31},Aib^{32}]NPY, [hPP$^{1-17}$,Ala^{31},Aib^{32}]hNPY, and [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31}, Aib^{32},Gln^{34}]hPP (Cabrele et al., 2000)} have been identified but, until now, no Y_1-selective agonist has been reported. Originally, [Leu^{31},Pro^{34}]-NPY had been described as Y_1-selective (Fuhlendorff et al., 1990). The Y_2 binding affinity of NPY was eliminated in [Leu^{31},Pro^{34}]-NPY by removing the C-terminal hexapeptide, which is known to be required for Y_2 binding, and substituting the C-terminal hexapeptide of PP. However, after identification of the Y_4 and Y_5 receptor subtypes, it was found that [Leu^{31},Pro^{34}]-NPY also has significant affinity and potency at these receptors (Gerald et al., 1996; Hu et al., 1996).

The identification of **1** and **2** as Y_1-selective agonists was based upon the results of an earlier study in which it was determined that substitution of the corresponding D-amino acid at either position 25 or 26 of NPY results in loss of Y_2 affinity but maintains Y_1 affinity (Boublik et al., 1989; Kirby et al., 1993a). **1** and **2** also inhibit forskolin-stimulated cAMP production in cells expressing the Y_1 receptor with EC_{50} values similar to that of NPY but are significantly less potent activators of the Y_2 receptor. The present study demonstrates that **1** and **2** are also selective for the Y_1 receptor relative to the Y_4 and Y_5 receptors. The loss of Y_2, Y_4, and Y_5 receptor binding upon changing the chirality of a single amino acid in the C-terminal α-helical segment of the molecule suggests that these receptors have more stringent requirements for the native conformation of this region of NPY than the Y_1 receptor. Work is in progress to further clarify these requirements.

The design of the internally truncated Y_1 agonists (**4**-**8**) was based on earlier observations that the putative β turn (amino acids 9–14) constituting the PP fold can be deleted without loss of Y_1 affinity, provided that the N- and C-terminal helices are held in close juxtaposition by a covalent linkage (Kirby et al., 1993b, 1995, 1997). Kirby et al. (1993b) found that a disulfide linkage between Cys^7 and Cys^{21} was superior to other interchain bridges examined for maintaining Y_1 affinity. **4** and **5** had very high Y_1 affinity and potency but also had high affinity and potency at the Y_4 and Y_2 receptors, respectively. Substitution of Pro^{34} in **5** to yield **6** reduced the affinity for Y_2 but, not unexpectedly, increased the affinity for Y_4. Because **2** is approximately 15-fold selective for Y_1 over Y_2 (K_i = 2.0 nM versus 29 nM), a D-His substitution in **5** was made to yield **7**. This modification did not result in the intended loss of affinity for Y_2 but did result in a significant loss of affinity for Y_4. Thus, both Pro^{34} and D-His^{26} were included in **8**, producing a peptide with excellent Y_1 selectivity and adequate Y_1 affinity and potency to be useful as a biological tool.

The stimulation of food intake by **1**, **2**, and **8** is the first demonstration that selective activation of the Y_1 receptor in animals expressing both the Y_1 and Y_5 receptors can induce feeding. The in vivo activity of both **1** and **2** is comparable with that obtained using equal doses of NPY. The stimulation of food intake observed after i.c.v. administration of **8** was more modest, and **4** failed to elicit food intake when administered at doses as high as 1 nmol. The failure of **4** to stimulate food intake was unexpected, because its K_i and EC_{50} values at the Y_1 receptor are lower than those of NPY. Rapid elimination, degradation, or poor solubility may cause the poor efficacy of both cyclized analogs tested in vivo, limiting their access to the receptor sites. The complete inhibition of **1**-stimulated food intake by the Y_1 selective antagonist 1229U91 confirms that the activity of **1** is not mediated by the Y_5 receptor. The 70% reduction in food intake measured after i.c.v. administration of 1229U91 alone suggests that Y_1 activation contributes to the spontaneous feeding and underscores the importance of the Y_1 receptor subtype in appetitive behavior.

The relative roles of the NPY Y_1 and Y_5 receptors in the regulation of food intake and energy expenditure are still unclear. The Y_1-selective agonists described here will be useful in characterizing more completely the role of the Y_1 receptor, not only in feeding behavior, but also in other physiological activities.

TABLE 2

Potencies of NPY, PP, and NPY analogs for the cloned human or rat NPY receptor subtypes.

The ability of NPY, PP, and various NPY analogs to inhibit forskolin-stimulated cAMP production in HEK 293 or CHO-K1 cells expressing one of the four human or rat NPY receptor subtypes was measured. cAMP assays were done in triplicate. The values are the mean ± S.E.M. of three determinations except where noted.

Peptide	K_i			
	Y_1	Y_2	Y_4	Y_5
	nM			
NPY	0.6 ± 0.1	2.2 ± 0.2	N.D.	2.5 ± 0.4
PP	N.D.	N.D.	0.04 ± 0.009	N.D.
1 [D-Arg^{25}]-NPY	0.2 ± 0.02	28.1 ± 5.0	577 ± 83	118.2 ± 22
2 [D-His^{26}]-NPY	0.5 ± 0.04	44.4 ± 6.4	1,669 ± 268	188 ± 58.4
3 [D-Arg^{25}, D-His^{26}]-NPY	13.6 ± 1.7	642 ± 208	10,632 ± 3,620	9,279 ± 1,440
4 Des-AA$^{10-17}$ [Cys^{7,21}, Pro^{34}]-NPY	0.3 ± 0.03	345 ± 237	5.0 ± 1.8	169 ± 21.9
5 Des-AA$^{11-18}$ [Cys^{7,21}, D-Lys^9 (Ac)]-NPY	0.5 ± 0.05	3.2 ± 0.4[a]	143 ± 47[a]	213 ± 20.8
6 Des-AA$^{11-18}$ [Cys^{7,21}, D-Lys^9 (Ac), Pro^{34}]-NPY	0.4 ± 0.16	270 ± 28.4	3.2 ± 0.34	348 ± 74.1
7 Des-AA$^{11-18}$ [Cys^{7,21}, D-Lys^9 (Ac), D-His^{26}]-NPY	5.3 ± 1.32	509 ± 239	>20,000	>20,000
8 Des-AA$^{11-18}$ [Cys^{7,21}, D-Lys^9 (Ac), D-His^{26}, Pro^{34}]-NPY	5.1 ± 0.97	>20,000	74.2 ± 18.6	>20,000

N.D., not determined.

[a] Mean ± S.D. (n = 2).

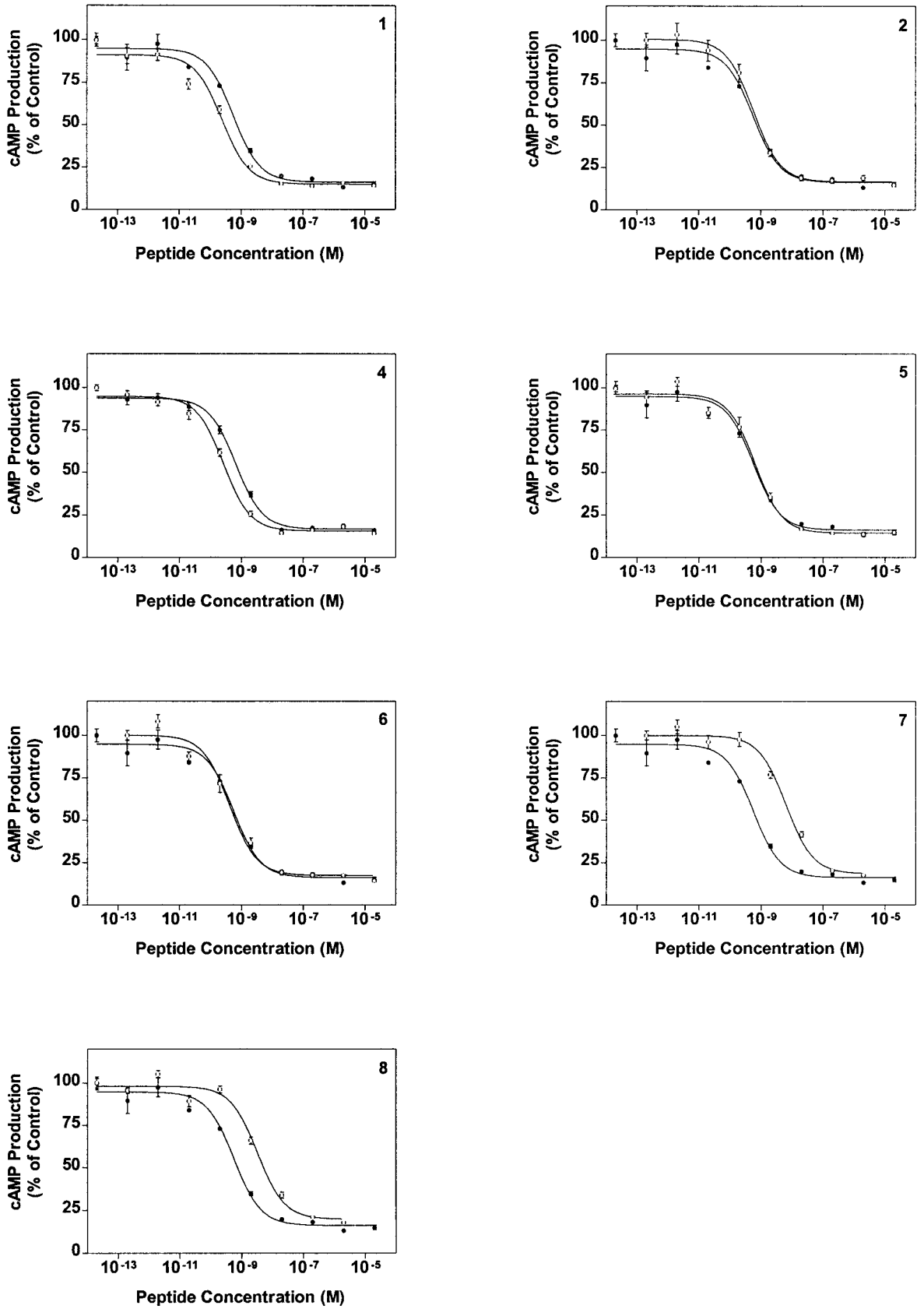

Fig. 1. Inhibition of forskolin-stimulated cAMP production by NPY and selective NPY Y_1 receptor agonists. HEK 293 cells expressing the human NPY Y_1 receptor were exposed to forskolin (5 μM) and NPY (●) and **1** [D-Arg^{25}]-NPY, **2** [D-His^{26}]-NPY, **4** Des-AA$^{10-17}$[Cys^{7,21},Pro^{34}]-NPY, **5** Des-AA$^{11-18}$[Cys^{7,21},D-Lys^{9}(Ac)]-NPY, **6** Des-AA$^{11-18}$[Cys^{7,21},D-Lys^{9}(Ac), Pro^{34}]-NPY, **7** Des-AA$^{11-18}$[Cys^{7,21},D-Lys^{9}(Ac), D-His^{26}]-NPY or **8** Des-AA$^{11-18}$ [Cys^{7,21},D-Lys^{9}(Ac), D-His^{26},Pro^{34}]-NPY (○) for 10 min. Intracellular cAMP levels were measured by radioimmunoassay. The values shown are the average ± S.E.M. of three experiments.

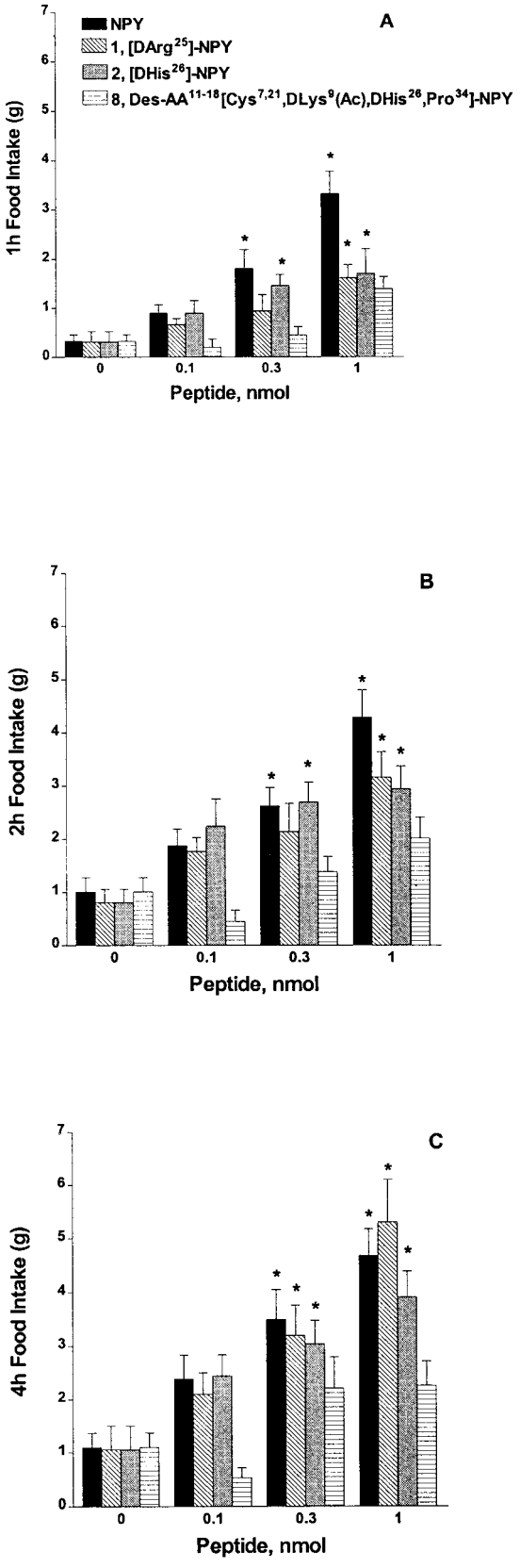

Fig. 2. Stimulation of food intake by NPY and selective NPY Y_1 receptor agonists. Cannulated Long Evans rats were injected i.c.v. with 0.9% NaCl (0 nmol) or peptide at doses of 0.1, 0.3 or 1 nmol. Food intake was measured 1 h (A), 2 h (B), and 4 h (C) after dosing. The values are the mean ± S.E.M. ($n = 6$). *$p < 0.05$; values are significantly different from the saline control group.

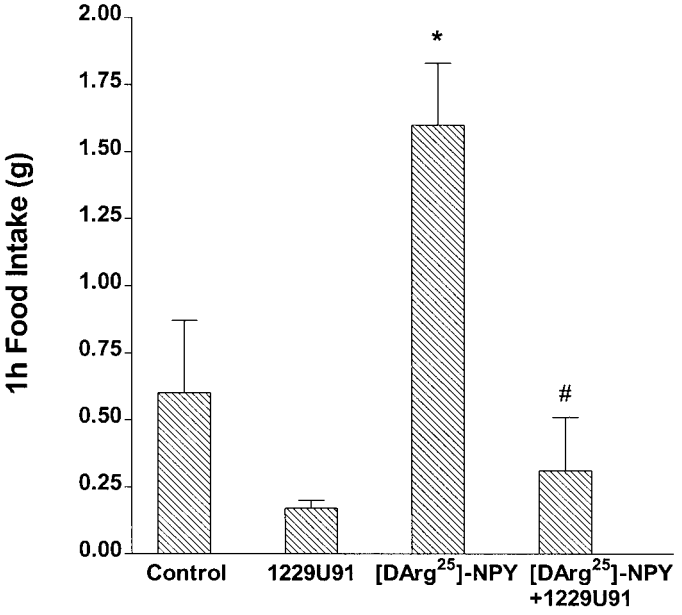

Fig. 3. Inhibition of [D-Arg^{25}]-NPY-stimulated food intake by 1229U91. Cannulated Long Evan rats were injected i.c.v. with 0.9% NaCl, 3 nmol of 1229U91, 0.3 nmol of **1**, or 3 nmol of 1229U91 and 0.3 nmol of **1**. Food intake was measured 1 h after dosing. The values are the mean ± S.E.M. ($n = 11$). *$p < 0.05$; value is significantly different from the saline control group. #$p < 0.001$; value is significantly different from **1** (0.3 nmol).

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