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Pharmacological characterization of the cloned neuropeptide Y y_6 receptor

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Abstract

Neuropeptide Y has potent appetite stimulating effects which are mediated by hypothalamic receptors believed to be of the neuropeptide Y Y_1 and/or neuropeptide Y Y_5 subtype. In mice, the neuropeptide Y y_6 receptor is also expressed in the hypothalamus, suggesting that it too may function as a feeding receptor in this species. Several laboratories have studied the pharmacology of the neuropeptide Y y_6 receptor, but their results are not in agreement. Using neuropeptide Y and a variety of peptide analogs and small molecule antagonists, we have determined that the pharmacology of the cloned mouse neuropeptide Y y_6 receptor is distinct from that of the other known neuropeptide Y receptors. The rank order of binding affinity for the mouse neuropeptide Y y_6 receptor is [(Ile,Glu,Pro,Dpr,Tyr,Arg,Leu,Arg,Tyr–NH_2)$_2$ cyclic (2,4′),(2′,4)-diamide] (1229U91) > human peptide YY = human, rat neuropeptide Y = human, rat neuropeptide Y-(2–36) = human, rat [Leu^{31}, Pro^{34}]neuropeptide Y > human, rat neuropeptide Y-(3–36) > human, rat neuropeptide Y-(13–36) > porcine (Cys^2)-neuropeptide Y-(1–4)-8-aminooctanoyl-(D-Cys^{27})-neuropeptide Y-(25–32) (C2–neuropeptide Y) > porcine [D-Trp^{32}]neuropeptide Y > rat pancreatic polypeptide = human pancreatic polypeptide. A similar rank order of potency is seen for inhibition of forskolin-stimulated cyclic AMP. The neuropeptide Y Y_5 receptor antagonist *trans*-naphthalene-1-sulfonic acid {4-[4-amino-quinazolin-2-ylamino)-methyl]-cyclohexylmethyl}-amide hydrochloride (CGP 71683A) and the neuropeptide Y Y_1 receptor antagonist ((*R*)-*N*^{2}-diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-argininamide) (BIBP3226) bind weakly to the neuropeptide Y y_6 receptor (K_i 2255 ± 197 nM and > 10,000 nM, respectively). Although the function of the neuropeptide Y y_6 receptor remains to be elucidated, its pharmacology is not consistent with a role in appetite regulation.

Keywords: Neuropeptide Y receptor; Neuropeptide Y y_6 receptor; Neuropeptide Y; Orexigenesis; 1229U91

1. Introduction

Neuropeptide Y is a 36-amino acid amidated peptide that is widely expressed in neurons, both centrally and peripherally. Neuropeptide Y has diverse physiological effects, including stimulation of feeding behavior (Heinrichs et al., 1998), regulation of metabolic rate (Billington and Levine, 1992), vasoconstriction (Lundberg et al., 1982), anxiolysis (Heilig et al., 1989), anti-convulsant activity (Vezzani et al., 1999) and regulation of ethanol consumption (Thiele et al., 1998). These effects are mediated via binding of neuropeptide Y to a family of G protein-coupled receptors. To date, four human receptor subtypes have been cloned (neuropeptide Y Y_1, Y_2, Y_4 and Y_5), and the existence of an neuropeptide Y Y_3 receptor has been hypothesized based upon its unique pharmacological profile (Balasubramaniam, 1997; Blomqvist and Herzog, 1997). A sixth neuropeptide Y receptor, neuropeptide Y y_6 (also referred to as Y_5 (Weinberg et al., 1996), PP2 (Gregor et al., 1996) and Y_{2b} (Matsumoto et al., 1996)), has been identified in mice and rabbits but is not expressed in primates or rats. The neuropeptide Y y_6 receptor gene in primates is a pseudogene containing a single base deletion in the sixth transmembrane domain that results in a truncated receptor (Gregor et al., 1996; Matsumoto et al., 1996; Rose et al., 1997). The primate gene is transcribed into mRNA, but a functional receptor apparently is not produced. A neuropeptide Y y_6 receptor

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gene has not been detected in rats (Burkhoff et al., 1998). The neuropeptide Y y_6 receptor gene has also been identified in hamster, chicken and cow, but its functional status in unknown (Matsumoto et al., 1996; Burkhoff et al., 1998).

Because of the role of neuropeptide Y in regulating food intake and body weight, the neuropeptide Y receptors are under investigation as therapeutic targets for the treatment of obesity. The use of neuropeptide Y receptor selective agonists and antagonists to study feeding behavior in rats has implicated the Y_1 and/or Y_5 receptor subtypes as ''feeding receptors'' (Criscione et al., 1998; Doods et al., 1995; Gerald et al., 1996; Hu et al., 1996; Ishihara et al., 1998; Kanatani et al., 1996; Kask et al., 1998; Matthews et al., 1997; Rudolf et al., 1994; Wieland et al., 1998). Similar conclusions have been drawn from studies using neuropeptide Y Y_1 or Y_5 receptor null mice (Pedrazzini et al., 1998; Marsh et al., 1998). It is currently unclear whether the neuropeptide Y y_6 receptor plays a role in feeding behavior in species in which it is expressed, such as the mouse. The use of several murine obesity models, including the leptin-deficient *ob/ob* mouse and diet-induced obese models (Campfield et al., 1996), as well as the use of neuropeptide Y transgenic or neuropeptide Y receptor knock-out mice (Pedrazzini et al., 1998; Marsh et al., 1998) has made it of interest to ascertain whether the neuropeptide Y y_6 receptor is involved in the neuropeptide Y-stimulated feeding response. The neuropeptide Y Y_1, Y_5 and y_6 receptors are all expressed in the hypothalamus (Gerald et al., 1996; Hu et al., 1996; Weinberg et al., 1996), a region known to regulate feeding behavior. Although two preliminary pharmacological studies of the mouse neuropeptide Y y_6 receptor have been reported, the results are not in agreement. Weinberg et al. (1996) reported a ''Y_1-like'' pharmacological profile for the neuropeptide Y y_6 receptor, while Gregor et al. (1996) reported a ''Y_4-like'' profile. In order to determine whether the neuropeptide Y y_6 receptor might function as a ''feeding receptor'' in the mouse we undertook a detailed pharmacological analysis of the receptor.

2. Materials and methods

2.1. Materials

Human embryonic kidney cells (HEK-293) were obtained from the American Type Culture Collection (Rockville, MD). Cell culture reagents, LipofectAMINE Reagent and Geneticin (G418) were purchased from Life Technologies (Gaithersburg, MD). Serum was obtained from ICN (Irvine, CA). Peptides were purchased from Bachem (King of Prussia, PA) or Peninsula (Belmont, CA) and are the human sequence unless otherwise noted. [(Ile,Glu, Pro,Dpr,Tyr,Arg,Leu,Arg,Tyr–NH_2)$_2$ cyclic (2,4′),(2′,4)-diamide] (1229U91) (also called GR231118 and GW1229) was synthesized by Anaspec (San Jose, CA). ((*R*)-N^2-diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-argininamide) (BIBP3226) was purchased from Peninsula Laboratories Belmont, CA). Pefabloc was purchased from Boehringer Mannheim (Indianapolis, IN), pcDNA3.1-Zeo and Zeocin from Invitrogen (Carlsbad, CA), and [^{125}I] porcine peptide YY, [^{125}I] human pancreatic polypeptide and cAMP [^{125}I] Flash Plate Assay from NEN (Boston, MA). All other chemicals were obtained from Sigma (St. Louis, MO). Trans-naphthalene-1-sulfonic acid {4-[4-amino-quinazolin-2-ylamino)-methyl]-cyclohexylmethyl}-amide hydrochloride (CGP 71683A) was synthesized at the Schering-Plough Research Institute (Criscione et al., 1998).

2.2. Cloning and expression of the neuropeptide Y receptors

HEK-293 cells expressing the rat neuropeptide Y Y_1, Y_4 or Y_5 receptor have been described previously (Parker et al., 1998). HEK-293 cells expressing the mouse neuropeptide Y y_6 receptor were isolated by previously described methods (Parker et al., 1998). Briefly, the mouse neuropeptide Y y_6 receptor cDNA (cloned as described in Parker et al., 1998) was subcloned into the pcDNA3.1-Zeo expression vector. HEK-293 cells were transfected with the expression vector by the use of LipofectAMINE Reagent according to the manufacturer's instructions. HEK-293 cells expressing the mouse neuropeptide Y y_6 receptor were selected in 200 μg/ml Zeocin, and individual cell lines were isolated as previously described (Parker et al., 1998). The levels of expression of the mouse neuropeptide Y y_6 receptor in the HEK-293 cell lines used in the study was 253 fmol/mg protein.

2.3. Cell culture

HEK-293 were grown in Dulbecco's Modified Eagle Medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 200 μg/ml Zeocin. Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air.

2.4. Radioligand binding assays

Membranes from cells expressing various neuropeptide Y receptors were prepared as described previously (Parker et al., 1998). The protein concentration was determined by the method of Bradford (1976). Radioligand binding assays were performed in 50 mM HEPES, pH. 7.2, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.1% bovine serum albumin containing 5–20 μg of membrane protein, 0.2 nM [^{125}I] peptide YY and the appropriate concentration of non-radiolabeled peptide in a total volume of 200 μl. Non-specific

binding was determined in the presence of 1 μM neuropeptide Y. The reaction mixtures were incubated for 90 min at room temperature then filtered through Millipore MAFC glass fiber filter plates presoaked in 0.5% (v/v) polyethyleneimine. The filters were washed twice with 150 μl of ice cold Dulbecco's phosphate-buffered saline, and radioactivity was measured in a Packard TopCount scintillation counter.

2.5. cAMP assay

HEK-293 cells expressing the neuropeptide Y y_6 receptor were plated 3 days prior to the assay in 96-well dishes at 1.5×10^4 cells/well. Immediately prior to the assay the confluent cell monolayers were washed once in Hank's Balanced Salt Solution (HBSS), then incubated for 20 min at 37°C in HBSS supplemented with 4 mM $MgCl_2$, 10 mM HEPES, 0.2% bovine serum albumin and 1 mM 3-isobutyl-1-methylxanthine. This solution was then replaced with the same solution containing the peptide of interest at concentrations ranging from 20 μM to 20 fM. The cells were incubated for 10 min at 37°C, the solution was then removed, and the cAMP was extracted in 75 μl of 100% ethanol. For determination of K_b values, the assay was performed as described above with the inclusion of the receptor antagonist of interest in the 20 min preincubation and the 10-min peptide incubation. The cAMP content of the samples was measured by radioimmunoassay (NEN Flash Plate).

2.6. Data analysis

Binding and cAMP data were analyzed by nonlinear regression using Prism (Graph Pad, San Diego, CA).

3. Results

The mouse neuropeptide Y y_6 receptor was transfected in HEK-293 cells and stable cell lines expressing the receptor were isolated. The stable cell line used in this study showed specific binding of [^{125}I] peptide YY with a K_d of 1.29 ± 0.12 nM and a B_{max} of 253 ± 5.3 fmol/mg of membrane protein ($n = 3$) (Fig. 1). No appreciable binding of [^{125}I] pancreatic polypeptide was observed, even at a relatively high radioligand concentration of 0.8 nM (data not shown).

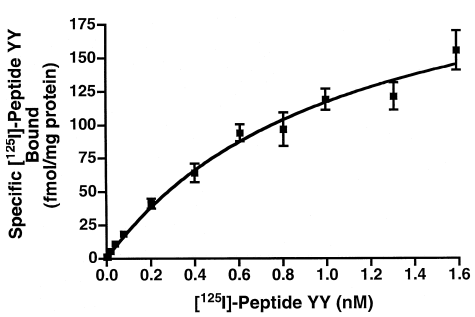

Fig. 1. Specific binding of [^{125}I]peptide YY to HEK 293 cells expressing the mouse neuropeptide Y y_6 receptor. Cell membranes were prepared as described in Section 2.4 and incubated with the indicated concentrations of [^{125}I] peptide YY in the presence or absence of 1 uM neuropeptide Y. The values shown are the average ± S.E.M. of three determinations.

Table 1
Affinities of neuropeptide Y, neuropeptide Y analogs and neuropeptide Y receptor antagonists for the mouse neuropeptide Y y_6 receptor
The neuropeptide Y y_6 receptor was expressed in HEK 293 cells. All binding assays were performed in duplicate using 5–20 μg membrane protein and 0.2 nM ^{125}I-peptide YY as described in Section 2.5. Values represent the average ± S.E.M. of three to six determinations.

Peptide	K_i (nM)
Neuropeptide Y	6.8 ± 2.3
Peptide YY	4.9 ± 2.0
Neuropeptide Y-(2–36)	8.5 ± 2.0
Neuropeptide Y-(3–36)	20.2 ± 9.7
Neuropeptide Y-(13–36)	43.6 ± 12.5
Neuropeptide Y-(18–36)	67.0 ± 8.9
Neuropeptide Y-(22–36)	122.3 ± 28.0
Neuropeptide Y-(26–36)	203.7 ± 20.7
[Leu^{31}, Pro^{34}]neuropeptide Y	9.7 ± 2.3
Human pancreatic polypeptide	1144 ± 315
Rat pancreatic polypeptide	700.7 ± 261.6
1229U91	0.8 ± 0.4
Porcine [D-Trp^{32}]neuropeptide Y	316.3 ± 104.3
C2–neuropeptide Y	79.8 ± 49.0
BIBP3226	> 10,000
CGP 71638A	2255 ± 197

The affinity of neuropeptide Y and various analogs for the cloned neuropeptide Y y_6 receptor is shown in Table 1. 1229U91 has the highest affinity for the neuropeptide Y y_6 receptor of all the peptides tested (Table 1) and also proved to be a very potent agonist for the neuropeptide Y y_6 receptor (Fig. 2, Table 2). Neuropeptide Y, peptide YY

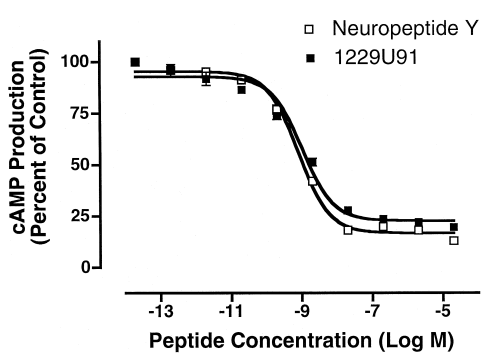

Fig. 2. 1229U91 is a neuropeptide Y y_6 receptor agonist. The ability of neuropeptide Y (open squares) or 1229U91 (closed squares) to block forskolin-stimulated cAMP production in HEK 293 cells expressing the mouse neuropeptide Y y_6 receptor was measured as described in Section 2.5. The values shown are the average ± S.E.M. of three experiments. The basal and forskolin-stimulated cAMP values in these experiments were 3.3 ± 0.3 pmol/ml and 84.9 ± 2.0 pmol/ml, respectively.

Table 2
Potencies of neuropeptide Y and neuropeptide Y analogs for the mouse neuropeptide Y y_6 receptor
The neuropeptide Y y_6 receptor was expressed in HEK 293 cells. EC_{50} values reflect the ability of the peptide (20 fM–20 μM) to inhibit forskolin-stimulated cyclic AMP production, measured using a radioimmunoassay. The data are expressed as the average ± S.E.M. of three determinations.

Peptide	EC_{50} (nM)
Neuropeptide Y	1.6 ± 0.3
Peptide YY	0.8 ± 0.1
Neuropeptide Y-(2–36)	1.4 ± 0.1
Neuropeptide Y-(3–36)	4.9 ± 0.8
Neuropeptide Y-(13–36)	16.1 ± 1.3
Neuropeptide Y-(18–36)	30.6 ± 8.6
Neuropeptide Y-(22–36)	132.2 ± 44.6
Neuropeptide Y-(26–36)	1002.4 ± 239.9
[Leu^{31}, Pro^{34}]neuropeptide Y	8.2 ± 3.9
Human pancreatic polypeptide	> 10,000
Rat pancreatic polypeptide	> 10,000
1229U91	0.8 ± 0.1
Porcine [D-Trp^{32}]neuropeptide Y	164.0 ± 16.2
C2–neuropeptide Y	324.9 ± 59.1

and [Leu^{31}, Pro^{34}]neuropeptide Y are also high affinity potent agonists for the neuropeptide Y y_6 receptor (Tables 1 and 2). The neuropeptide Y y_6 receptor exhibits a modest dependence on the *N*-terminal region of neuropeptide Y. Neuropeptide Y and neuropeptide Y-(2–36) are high affinity agonists, but progressive truncation of the molecule (neuropeptide Y-(3–36),-(13–36),-(18–36),-(22–36) and -(26–36) results in increasingly greater loss of both affinity and potency (Tables 1 and 2). The neuropeptide Y y_6 receptor has low affinity for human pancreatic polypeptide, rat pancreatic polypeptide, the neuropeptide Y Y_2 receptor-selective ligand porcine (Cys^2)-neuropeptide Y-(1-4)-8-aminooctanoyl-(D-Cys^{27})-neuropeptide Y-(25–32) (C2–neuropeptide Y) (McLean et al., 1990), and the neuropeptide Y Y_5 selective ligand [D-Trp^{32}]neuropeptide Y. Human pancreatic polypeptide and rat pancreatic polypeptide have no agonist activity at the neuropeptide Y y_6 receptor, whereas C2–neuropeptide Y and [D-Trp^{32}]neuropeptide Y have measurable agonist activity but are not particularly potent (Table 2).

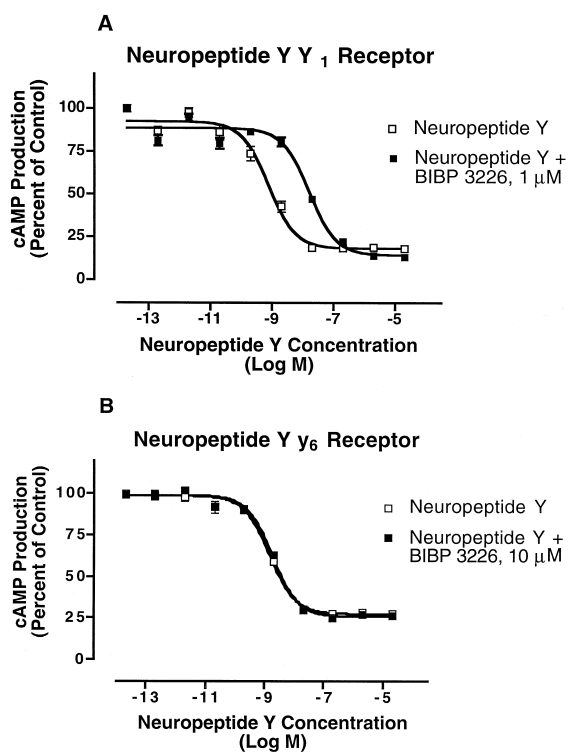

Fig. 3. Effect of the neuropeptide Y Y_1 receptor antagonist BIBP3226 on the functional response of the neuropeptide Y Y_1 (A) and neuropeptide Y y_6 (B) receptors. The apparent K_b value of BIBP3226 is 54.8 nM for the neuropeptide Y Y_1 receptor and > 10,000 nM for the neuropeptide Y y_6 receptor. EC_{50} and K_b values were determined as described in Section 2.5. The values shown are the average ± S.E.M. of three experiments. The basal and forskolin-stimulated cAMP concentrations were 2.1 ± 0.2 and 57.9 ± 4.1 pmol/ml (neuropeptide Y Y_1 receptor) and 3.9 ± 0.5 and 123.6 ± 10.1 pmol/ml (neuropeptide Y y_6 receptor).

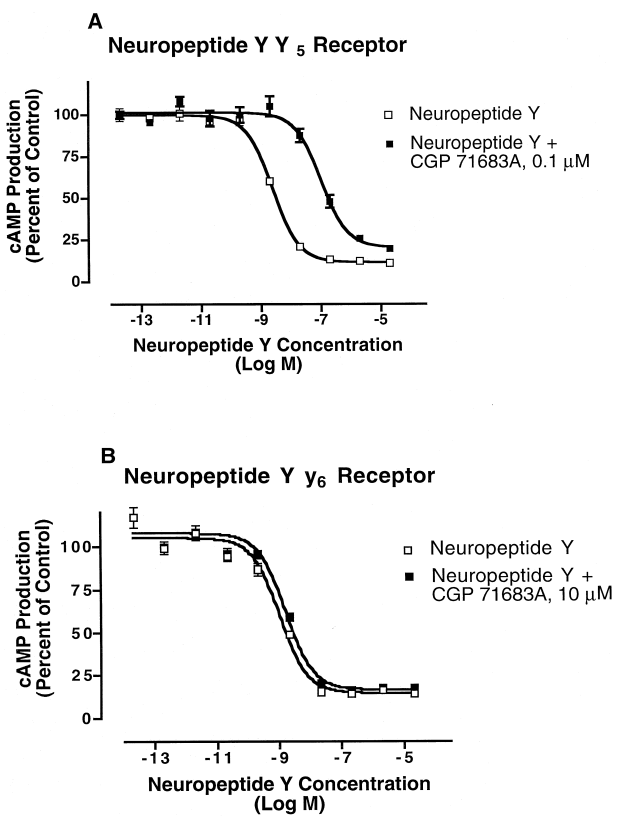

Fig. 4. Effect of the neuropeptide Y Y_5 antagonist CGP 71683A on the functional response of the neuropeptide Y Y_5 (A) and neuropeptide Y y_6 (B) receptors. The apparent K_b value of CGP 71683A is 2.5 nM for the neuropeptide Y Y_5 receptor and > 10,000 nM for the neuropeptide Y y_6 receptor. EC_{50} and K_b values were determined as described in Section 2.5. The values shown are the average ± S.E.M. of three experiments. The basal and forskolin-stimulated cAMP concentrations were 1.5 ± 0.1 and 51.2 ± 1.4 pmol/ml (neuropeptide Y Y_5 receptor) and 3.7 ± 0.5 and 120.6 ± 9.4 pmol/ml (neuropeptide Y y_6 receptor).

Two non-peptide neuropeptide Y receptor antagonists that have been shown to modulate feeding behavior were also tested for activity at the neuropeptide Y y_6 receptor. The neuropeptide Y Y_1 receptor antagonist BIBP3226 does not block binding of ^{125}I-peptide YY to the neuropeptide Y y_6 receptor ($K_i > 10{,}000$ nM) and has no apparent effect on neuropeptide Y inhibition of forskolin-stimulated cAMP production at concentrations as high as 10 μM (Fig. 3). The selective neuropeptide Y Y_5 receptor antagonist CGP 71683A binds the neuropeptide Y y_6 receptor with a K_i of 2255 nM and does not perceptibly block neuropeptide Y inhibition of forskolin-stimulated cAMP production when tested at 10 μM (Fig. 4).

4. Discussion

The hypothalamus has long been known to regulate feeding behavior, and Stanley et al. (Stanley et al., 1985; Stanley and Leibowitz, 1985) demonstrated that this is one site of action for the orexigenic effects of neuropeptide Y. The neuropeptide Y Y_1, Y_2, Y_5 and y_6 receptors are all expressed in the hypothalamus. Comparison of the pharmacological properties of the Y_1 and Y_5 receptors with the pharmacology of the receptor mediating neuropeptide Y-induced feeding implicate these two receptor subtypes as mediators of neuropeptide Y feeding effects (Blomqvist and Herzog, 1997; Statnick et al., 1998; Wahlestedt and Reis, 1993). Similar conclusions have been reached in studies of mice lacking the Y_1 and Y_5 receptors (Marsh et al., 1998; Pedrazzini et al., 1998). In contrast, the pharmacology of the Y_2 receptor suggests that this receptor is not involved in neuropeptide Y-induced feeding. The pharmacological properties of the mouse neuropeptide Y y_6 receptor have been controversial (Weinberg et al., 1996; Gregor et al., 1996). Although the sequence of the mouse neuropeptide Y y_6 receptors cloned by Weinberg et al. and by Gregor et al. are identical, the rank order of binding affinity of neuropeptide Y and several related peptides reported by these authors are dissimilar, with the most notable differences being the affinities of neuropeptide Y (4.3 nM vs. 638 nM) and of human pancreatic polypeptide (> 1000 vs. 0.057 nM). Hence, the purpose of the present study was to characterize the pharmacology of the mouse neuropeptide Y y_6 receptor and to determine if the neuropeptide Y y_6 receptor might mediate neuropeptide Y-induced feeding in the mouse.

The pharmacological properties of the neuropeptide Y y_6 receptor most closely resemble those of the neuropeptide Y Y_1 receptor. The rat neuropeptide Y Y_1 and mouse y_6 receptors share 56% amino acid sequence similarity overall, a higher degree of similarity than is shared among any of the other neuropeptide Y receptor subtypes (Gregor et al., 1996; Matsumoto et al., 1996; Weinberg et al., 1996). Both the Y_1 and y_6 receptors bind the dimeric nonapeptide 1229U91 with high affinity. However, 1229U91 is an agonist for the neuropeptide Y y_6 receptor but an antagonist for the neuropeptide Y Y_1 receptor. This peptide is also a high affinity agonist for the neuropeptide Y Y_4 receptor (Parker et al., 1998; Schober et al., 1998). Similar to the Y_1 and Y_5 receptors, the y_6 receptor subtype displays a dependence on the amino terminus of neuropeptide Y for binding and functional activation (neuropeptide Y = neuropeptide Y-(2–36) > neuropeptide Y-(3–36) > neuropeptide Y-(13–36) > neuropeptide Y-(18–36) > neuropeptide Y-(22–36) > neuropeptide Y-(26–36), although the requirement for the N-terminal amino acids is less stringent for the y_6 subtype than for the Y_1 subtype. Unlike the neuropeptide Y Y_2 receptor (Gehlert, 1994; Blomqvist and Herzog, 1997) the y_6 subtype has a low affinity for C2–neuropeptide Y and neuropeptide Y-(13–36) and a high affinity for [Leu^{31},Pro^{34}]neuropeptide Y. The high affinity of [Leu^{31},Pro^{34}]neuropeptide Y for the y_6 receptor subtype confirms that this peptide is non-selective. The neuropeptide Y y_6 receptor subtype has a low affinity for the neuropeptide Y Y_5 receptor-selective agonist [D-Trp^{32}]neuropeptide Y. The neuropeptide Y y_6 receptor binds both human and rat pancreatic polypeptide weakly, thus distinguishing this receptor from the neuropeptide Y Y_4 and Y_5 receptor subtypes. This result contradicts the finding of Gregor et al. (1996) who reported that the neuropeptide Y y_6 binds both rat pancreatic polypeptide and human pancreatic polypeptide with high affinity. The reason for this discrepancy is not clear. The binding affinities reported here for neuropeptide Y and related peptides are similar to those reported by Weinberg et al. (1996). Also, with the exception of neuropeptide Y-(13–36), they are similar to those reported by Matsumoto et al. (1996) for the rabbit neuropeptide Y Y_{2b} receptor. The relatively high affinity of the neuropeptide Y Y_2 receptor-selective ligand neuropeptide Y-(13–36) for the rabbit neuropeptide Y Y_{2b} receptor compared to the mouse neuropeptide Y y_6 receptor (5.0 nM vs. 43.6 nM) may be due to differences in the predicted amino acid sequences of these orthologues.

Several aspects of the pharmacology of the neuropeptide Y y_6 receptor suggest that this receptor does not mediate feeding behavior in the mouse. Two neuropeptide Y-related peptide agonists which stimulate feeding in rats when administered i.c.v., human pancreatic polypeptide and [D-Trp^{32}]neuropeptide Y, have relatively low affinity and minimal functional activity at the neuropeptide Y y_6 receptor. The neuropeptide Y Y_1 receptor antagonist 1229U91 inhibits food intake in mice but is a potent agonist for the neuropeptide Y y_6 receptor. Similarly, the neuropeptide Y Y_1 receptor antagonist BIBP3226 and the neuropeptide Y Y_5 receptor antagonist CGP 71683A inhibit food intake in rats but have no activity at the neuropeptide Y y_6 receptor. Although it is possible that the neuropeptide Y y_6 receptor could act in concert with another neuropeptide Y receptor to mediate feeding, data obtained using neuropeptide Y Y_5 receptor null mice sug-

gest this is not the case. In these mice administration of the neuropeptide Y Y_1-selective antagonist 1229U91 completely eliminated food intake in response to i.c.v. administration of neuropeptide Y, suggesting that neuropeptide Y-stimulated feeding is mediated entirely by the neuropeptide Y Y_1 and Y_5 receptors. Although the role of the neuropeptide Y y_6 receptor remains to be elucidated, its pharmacology suggests that it is not involved in appetite regulation.

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