0026-895X/96/020224-05$3.00/0

MOLECULAR PHARMACOLOGY, 49:224–228 (1996).

ACCELERATED COMMUNICATION

Expression Cloning of a Human Brain Neuropeptide Y Y2 Receptor

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Received October 26, 1995; Accepted November 8, 1995

SUMMARY

The 36-amino acid peptide, neuropeptide Y (NPY), is a member of a peptide family that includes the endocrine peptides, peptide YY (PYY), and pancreatic polypeptide (PP). NPY receptors have been broadly subdivided into postsynaptic Y1 receptors and presynaptic Y2 receptors based on the preference of Pro^{34}-substituted analogues for the Y1 receptors and carboxyl-terminal fragments for the Y2. A Y1 receptor has been cloned, and this receptor appears to mediate several effects of NPY, including vasoconstriction and anxiolysis in animal models. We report the cloning of a human brain Y2 receptor from a human brain library. Pools of clones were transiently expressed in COS-1 cells, and ^{125}I-PYY binding pools were identified by autoradiography. After a single positive pool was detected in the original screening, a single clone was isolated by four rounds of sequential enrichment. The clone encoded a 381-amino acid protein of the heptahelix (seven TM) type. Amino acid identity of this receptor with the Y1 receptor was 31% overall with 40% identity in the TM regions. Comparison with the human PP1 receptor indicated 33% overall amino acid identity with 42% identity in the TM regions. Pharmacologically, the receptor exhibited high affinity for NPY, PYY, and carboxyl-terminal fragments of NPY and PYY. In addition, Pro^{34}-substituted analogues had very low affinity. With the use of Northern blot analysis, high levels of Y2 mRNA were detected in a variety of brain regions with little expression in peripheral tissues. Thus, the receptor protein has the pharmacological properties and distribution of the human Y2 receptor.

NPY is a 36-amino acid peptide that was first isolated from porcine brain. It is one of the most abundant peptides in brain (1) and is a member of a family of closely related peptides (2) that includes PYY and PP. All of these peptides have 36 amino acids, carboxyl-terminal tyrosine amides, and a characteristic structure consisting of a type II prolyl/prolyl helix followed by a β turn and an α helical region (3). This structure serves to place the amino- and carboxyl-terminal regions in close proximity. In the central nervous system, NPY stimulates food intake, reduces blood pressure, causes neuroendocrine alterations, changes circadian rhythms, and affects memory processing (for reviews, see Refs. 4 and 5). In the peripheral nervous system, NPY is co-released from sympathetic nerves along with norepinephrine and appears to play an important role in cardiovascular regulation (6). NPY produces its biological effects by interacting with at least two distinct receptor subtypes characterized by the differential activity of peptide agonists (for review, see Ref. 4). The Y1 receptor binds both full-length NPY and PYY but also recognizes Pro^{34}-substituted analogues (7). The Y2 receptor also recognizes both NPY and PYY but is less sensitive to Pro^{34}-substituted analogues. In addition, carboxyl-terminal fragments of NPY and PYY are potent agonists at Y2 but have much lower affinity at Y1 (8). Additional subtypes of NPY receptors have been proposed, such as the Y3 receptor, which has preferential affinity for NPY over PYY (9), and a distinct Y1 variant that mediates the feeding response to centrally administered NPY (10, 11).

Molecular cloning has revealed a G protein-coupled receptor that was initially considered to be an orphan receptor (12) but later was found to be the Y1 subtype (13–15). This receptor displays a pharmacology and brain distribution that are consistent with the properties of the Y1 receptor. Recently, using a homology-based approach, we identified a human

The nucleotide sequence reported has been deposited in the Genbank data base (accession no. U42766).

ABBREVIATIONS: NPY, neuropeptide Y; hNPY, human neuropeptide Y; pNPY, porcine neuropeptide Y; PYY, peptide YY; hPYY, human peptide YY; pPYY, porcine peptide YY; PP, pancreatic polypeptide; hPP, human pancreatic polypeptide; TM, transmembrane; SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

PP-preferring receptor that we have named PP1 (16). This receptor had 43% amino acid identity with the human Y1 receptor and was found primarily in peripheral tissues. This receptor has very high affinity for PP (K_i = 16 pM) with 10-fold lower affinity for PYY and 1000-fold lower affinity for NPY. The remaining member of the known receptor family is the Y2 receptor. This receptor is found in a variety of brain regions (17, 18) and is expressed in several human-derived cell lines such as the SMS-KAN (19). Although extensive pharmacological and biochemical characterization of this receptor has been performed, successful cloning of this receptor subtype by the homology approach has been difficult. To clone and express the human Y2 receptor, we used an expression cloning technique to identify cDNA encoding a Y2 receptor from a human brain expression library.

Materials and Methods

Expression cloning. The human brain cDNA library in the expression vector pcDNAI (Clonetech, Palo Alto, CA) was amplified into 58 pools of ~5000 clones. Average insert size of the library was 1.4 kb. Plasmid DNA was prepared by the alkaline lysis technique and transfected using the DEAE-dextran technique into African green monkey kidney cells (COS-1) in suspension. The cells were plated onto plastic chamber slides and grown for 48 hr after transfection. Subsequently, the chambers were removed, and the cells were rinsed with phosphate-buffered saline. The slides were incubated with 100 pM ^{125}I-pPYY in a modified Krebs-phosphate buffer containing 0.1% bacitracin and 0.4% bovine serum albumin as previously described (4). A second set of slides were incubated with the addition of 1 μM NPY to assess nonspecific binding. After incubation and rinsing, the labeled slides were placed against a sheet of Hyperfilm-[^{3}H] (Amersham, Arlington Heights, IL) in X-ray cassettes and exposed for 3 days. A positive pool was divided into subpools and screened repetitively until a single clone, DSL9, was identified. The nucleotide sequence of both strands of DSL9 was determined using the dideoxy chain termination method. Sequence analysis was performed using the GCG (20) software package.

Binding assay. Binding assays were performed with crude membranes isolated from COS-1 cells transiently transfected with DSL9 using the DEAE-dextran technique. For comparison, crude membranes were used from a stable cell line transfected with the human Y1 receptor (AV12-Y1) and the Y2-containing SMS-KAN cell line. Cells were scraped from the culture plates into phosphate-buffered saline and pelleted in tubes. The homogenate binding studies were conducted as previously described (18). The cell pellets were resuspended using a glass homogenizer in a 25 mM HEPES, pH 7.4, buffer containing 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 2 g/l bacitracin. Cell membranes were incubated in a final volume of 200 μl containing various concentrations of ^{125}I-pPYY (specific activity, 2200 Ci/mmol, DuPont-NEN, Boston, MA) for 2 hr at room temperature. Nonspecific binding was defined as the amount of radioactivity remaining bound after incubation in the presence of 1 μM hNPY. In pharmacological studies, various concentrations of peptides (Peninsula, Belmont, CA, or Bachem, King of Prussia, PA) were included in the incubation mixture. Incubations were terminated by rapid filtration through GF/C filters (Wallac, Gaithersburg, MD) that had been presoaked in 0.3% polyethyleneimine (Sigma Chemical Co., St. Louis, MO) with the use of a TOMTEC (Orange, CT) cell harvester. The filters were washed with 5 ml of 50 mM Tris, pH 7.4, at 4° and rapidly dried at 60°. The dried filters were treated with MeltiLex A (Wallac) melt-on scintillator sheets, and the radioactivity retained on the filters was counted with the use of the Wallac 1205 Betaplate counter. The results were analyzed using the Prism software package (Graphpad, San Diego, CA). Protein concentrations were measured using Coomassie Protein Assay Reagent (Pierce, Rockford, IL) with bovine serum albumin used as standards.

Northern hybridization. Northern membranes containing mRNA from various human brain regions and organs were purchased from Clonetech. The membranes were prehybridized for 4 hr at 42° in 5× standard saline/phosphate/EDTA buffer, 10× Denhardt's solution, 100 μg/ml sheared salmon sperm DNA, 50% formamide, and 2% SDS. This was followed by hybridization overnight at 42° in the same buffer containing 2 × 10^6 cpm of a nick-translated (BRL, Gaithersburg, MD) fragment containing the entire coding region of the clone DSL9. The membranes were washed three times (2× standard saline citrate, 0.05% SDS at room temperature) and once for 40 min at high stringency (0.1× standard saline citrate, 0.1% SDS at 50°). The blots were visualized using a Phosphorimager (Molecular Dynamics, Sunnydale, CA).

Results and Discussion

From a screen of ~300,000 clones from the human brain expression library, one pool was identified as a positive (Fig. 1). Four rounds of subpool screening led to the isolation of a full-length clone, DSL9 (Fig. 1). Subsequently, the full-length clone was expressed transiently in COS-1 cells and characterized by radioligand binding. ^{125}I-pPYY bound to a single, high affinity site with a K_d of 50 ± 6 pM and a B_{max} of 3600 ± 600 fmol/mg protein (four experiments) (Fig. 2). Nontransfected or mock transfected cells did not exhibit specific bind-

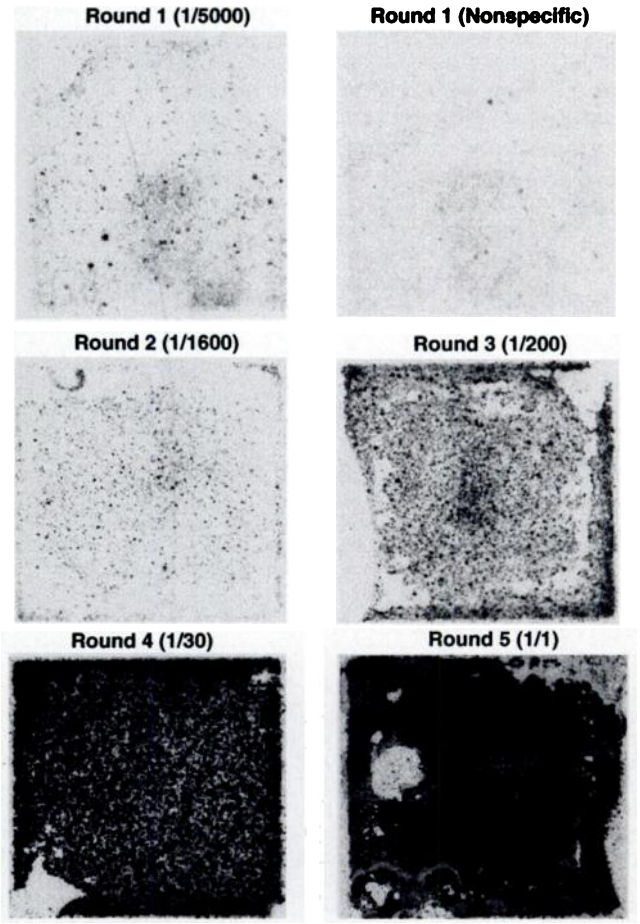

Fig. 1. Enrichment of ^{125}I-pPYY binding clone from a human brain expression library is shown in autoradiograms from cells transfected with various clone pools and labeled with ^{125}I-pPYY. Round 1 (top left) indicates the first positive pool identified. The enrichment of the clone by the subsequent subdivision is shown by the increased binding in rounds 2, 3, and 4.

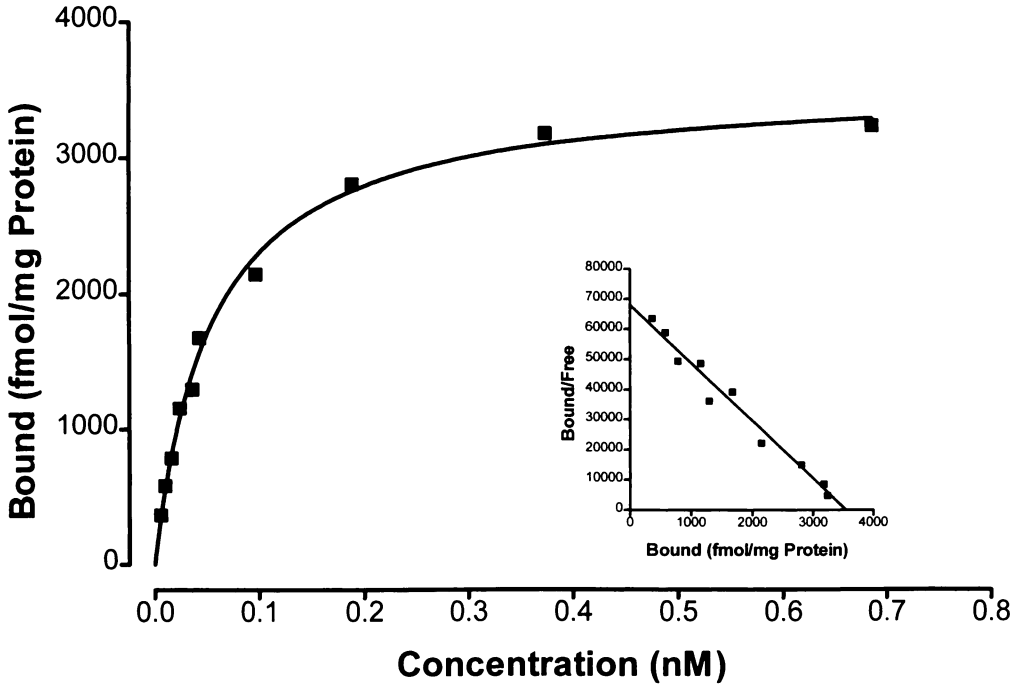

Fig. 2. Saturation analysis of ^{125}I-pPYY to COS-1 membranes from cells transiently transfected with clone DSL9. The figure shows a representative experiment. Values from four experiments are given in Results.

ing of ^{125}I-pPYY (data not shown). The pharmacology of the binding was evaluated by competing the radioligand from the receptor with various peptides. hNPY and hPYY were the most potent inhibitors of binding, whereas hPP did not inhibit binding at concentrations of up to 1 μM (Table 1). Several carboxyl-terminal fragments of NPY and PYY were also potent inhibitors of binding. pNPY2–36 and pNPY3–36 inhibited binding with slightly lower affinities than the intact peptides. pNPY13–36 had ~6-fold lower affinity compared with hNPY with a K_i of 1.8 nM. Fragments of PYY had somewhat higher affinity compared with NPY fragments. hPYY3–36 inhibited binding with a K_i of 0.21 nM, and pPYY13–36 had a K_i of 0.38 nM. On the other hand, the Pro^{34}-substituted analogues of NPY and PYY were significantly less potent at this receptor. p[Leu^{31},Pro^{34}]NPY, h[Leu^{31},Pro^{34}]PYY, and h[Pro^{34}]PYY inhibited binding at high nanomolar concentrations. In addition, the nonpeptide Y1 selective antagonist BIBP3226 (21) and the "feeding selective" antagonist [D-Trp^{32}]NPY (22) were relatively inactive. These data are similar to those described for the Y2 receptor expressed in human brain and cell lines specifically expressing the Y2 receptor. In Table 1, data obtained using the Y2-containing cell line, SMS-KAN (19), are very similar to those obtained with the human brain Y2 receptor. However, these data are in sharp contrast to those for the hY1 receptor. At this receptor, NPY and PYY are also equipotent, and PP exhibits some inhibition of binding at higher concentrations (Table 1). Unlike Y2, the carboxyl-terminal fragments of NPY and PYY had relatively low affinity for Y1, and the Pro^{34}-substituted analogues had affinity similar to the native peptides. The binding profile of the human Y2 receptor differs significantly from the hPP1 receptor, which displays low picomolar affinity for hPP (16), whereas at the human Y2 receptor, this peptide does not appreciably inhibit binding at 1 μM concentrations.

TABLE 1

Pharmacological characterization of AV12-Y1, SMS-KAN, and COS-1-DSL9 cells

Membranes from the indicated cell lines were incubated with ^{125}I-pPYY and various concentrations of the indicated peptides and compound as described in Methods. Data represent the mean ± standard error of four determinations performed in duplicate.

Inhibitor	K_i		
	AV12-Y1	SMS-KAN	COS-1-DSL9
		nM	
hNPY	0.18 ± 0.02	0.25 ± 0.02	0.30 ± 0.02
hPYY	0.14 ± 0.01	0.14 ± 0.01	0.17 ± 0.01
hPP	19 ± 1	>1000	>1000
pNPY	0.086 ± 0.005	0.21 ± 0.01	0.47 ± 0.02
pNPY2–36	0.84 ± 0.03	0.27 ± 0.04	0.50 ± 0.03
pNPY3–36	9.0 ± 1.0	0.59 ± 0.03	0.65 ± 0.01
pNPY13–36	12.5 ± 0.2	1.3 ± 0.1	1.8 ± 0.3
hPYY3–36	13.3 ± 0.7	0.24 ± 0.05	0.21 ± 0.03
pPYY13–36	9.5 ± 1.3	0.30 ± 0.04	0.38 ± 0.01
p[Leu^{31},Pro^{34}]NPY	0.13 ± 0.01	390 ± 50	760 ± 20
h[Leu^{31},Pro^{34}]PYY	0.16 ± 0.0.04	500 ± 10	700 ± 190
h[Pro^{34}]PYY	0.069 ± 0.004	320 ± 20	480 ± 130
BIBP3226	9.7 ± 1.1	>1000	>1000
[D-Trp^{32}]NPY	>1000	160 ± 10	160 ± 20

The 3747-nucleotide cDNA encodes a deduced 381-amino acid protein with an estimated molecular mass of 42 kDa (Fig. 3). This size is in agreement with a recent report of the purified porcine Y2 receptor, which has a molecular mass of 42 kDa after treatment with deglycosylating enzymes (23). Hydropathy analysis indicates the presence of seven putative TM domains, consistent with membership in the family of G protein-coupled receptors. There is a single N-linked glycosylation site in the amino-terminal region and a cysteine in the carboxyl-terminal region that may be involved in palmitoylation at residue 343. The Y2 receptor exhibits relatively low sequence identity with the Y1 receptor, with 31% overall identity and 56% similarity. Compared with the PP1 receptor, the Y2 receptor has 33% overall identity and 54% simi-

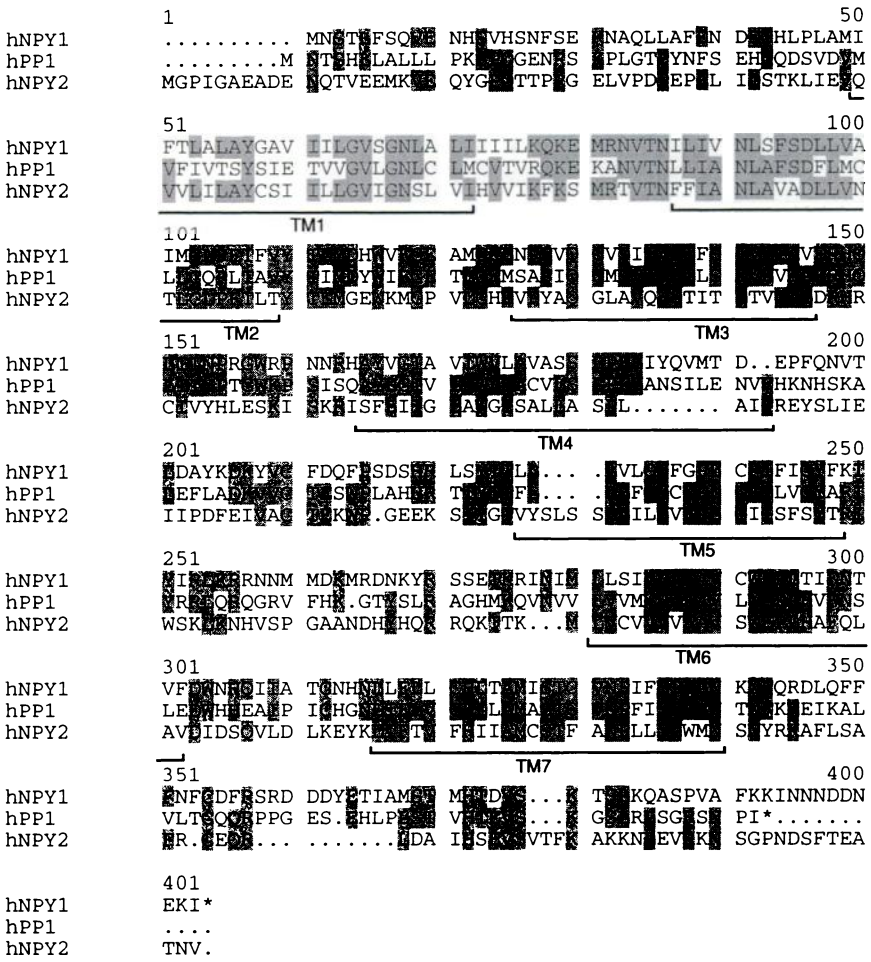

Fig. 3. Amino acid sequence of the human brain Y2 receptor. *Bracketed areas*, putative TM regions. The amino acid sequences of the human Y1 and PP1 receptors are included for comparison. *Shaded areas*, regions with identity between the three sequences.

larity. When examining the TM regions, a relatively low overall sequence identity was observed compared with the Y1 (40%) and PP1 (42%) receptors. In the Y1 receptor, TM1 and TM6 display the highest level of amino acid identity, with Y2 at ~50% identity; TM4 has the lowest, with 18%. TM6 and TM7 of the PP1 receptor showed the highest identity with Y2 at 59% and 56%, respectively, with TM3 having the lowest with 27% identity. These data suggest that the Y2 represents a novel or distantly related receptor family from the Y1/PP1 family. Further study and molecular cloning of the Y2 receptor from several species will be necessary to adequately explore this hypothesis.

When we examined the overall sequence of the Y2 receptor and compared it with the Y1 and PP1 receptors, several interesting features were apparent. The only stretch of conserved amino acids across all three receptors was in TM6, with the motif VVXFAVXWLPL. The sequence ERHQLIINP, which is conserved among the Y1 and PP1 receptors from several species (2, 16), is not conserved in the Y2 receptor. The Y2 sequence also differs from the Y1 and PP1 in that it has a single amino-terminal glycosylation site, whereas the others have three. Of the four extracellular cysteines found in Y1 and PP1, only two are conserved in the Y2 with C123 and C203. The cysteines found in the amino-terminal regions and in extracellular loop 3 are missing. Using site-directed mutagenesis, Walker *et al.* (24) identified four acidic amino acid residues that appear to be critical for NPY binding to the Y1 receptor: D104, D194, D200, and D287. In the hPP1 receptor, these amino acids are conserved with the negatively charged amino acids D103, D195, E201, and D287. The Y2 shows some conservation but has one important difference. In these positions, Y2 has the substitution G113, the conservative substitutions E205 and E210, and the conserved D292. Interestingly, the Y2 receptor has high affinity for carboxyl-terminal fragments of NPY and PYY, whereas the Y1 receptor does not (Table 1). Therefore, amino acids in the Y1 receptor that recognize the amino-terminal regions of NPY and PYY would not be expected to be conserved in the Y2 receptor; this will be addressed in future mutagenesis studies.

The Y2 receptor is believed to be presynaptic on peripheral and central neurons, and stimulation by NPY results in a decrease in the release of neurotransmitters and decreased cellular excitability (25). Northern blot analysis of poly(A)$^+$ RNA from human brain regions and peripheral tissues revealed a 4-kb hybridizing band (Fig. 4). A number of brain regions expressed a relatively high level of mRNA encoding for Y2; cerebral cortex, amygdala, and hippocampus. Lower levels were seen in the caudate, subthalamic nucleus, and medulla. Very low levels were seen in the substantia nigra, thalamus, and putamen. No hybridization could be seen in the cerebellum and spinal cord. This mRNA distribution is similar to the described distribution of the Y2 receptor binding in the human brain (26). High levels of Y2 binding are found in the hippocampus and the amygdala, whereas moderate levels of Y2 are found in the cerebral cortex, substantia nigra, and caudate nucleus. In a broad panel of human peripheral tissues, a few organs containing mRNA were de-

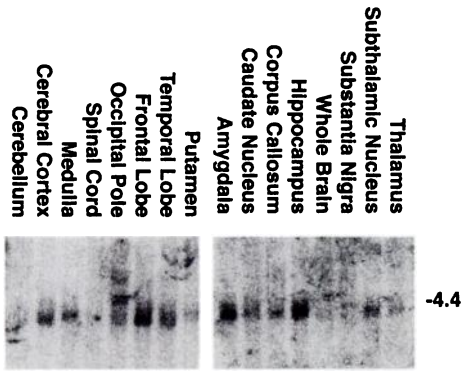

Fig. 4. Northern blot analysis of mRNA from human brain regions and peripheral tissues. Each lane contains 2 μg of poly $(A)^+$ RNA. The location of the 4.4-kb molecular weight marker is indicated.

tected, including a low level of expression in the small intestine (data not shown). Most peripheral tissues displayed no detectable hybridization, including the spleen, thymus, prostate, and testis. These data suggest that the Y2 receptor is predominantly expressed in the human brain. A Southern blot had a single band, indicating that a single gene encoding the Y2 receptor is found in humans (data not shown). Interestingly, peripheral postsynaptic Y2 receptors have been described in the rat vasculature (27) and porcine spleen (28). Further study will be necessary to determine whether the Y2 receptor described in these studies is identical to that described here.

In conclusion, we identified a clone from a human brain library that binds NPY and PYY with high affinity. This receptor has high affinity for carboxyl-terminal fragments of these peptides and much lower affinity for Pro^{34}-substituted analogues. Thus, this receptor has a pharmacological profile consistent with a Y2 receptor subtype.

During preparation of this manuscript, an article was published describing the cloning of a Y2 receptor from SMS-KAN cells (29). The clone described in that article has a single nucleic acid difference in the coding regions, resulting in A134V.

Acknowledgments

The authors thank Stan Burgett and Paul Rosteck at the Eli Lilly technology core facility for sequencing DSL9 and Maverick Ulmer for preparing the DNA pools from the human brain library. We also thank Paul Burnett and Nancy Mayne (Eli Lilly) for providing AV12-Y1 cells.

References

1. O'Donohue, T. L., B. M. Chronwall, R. M. Pruss, E. Mezey, J. Z. Kiss, L. E. Eiden, V. J. Massari, R. E. Tessel, V. M. Pickel, D. A. Dimaggio, A. J. Hotchkiss, W. R. Crowley, and Z. Zukowska-Grojec. Neuropeptide Y and peptide YY in neuronal and endocrine systems. *Peptides* **6:**755–768 (1985).
2. Larhammar, D., A. G. Blomquist, and C. Söderberg. Evolution of neuropeptide Y and its related peptides. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* **106:**743–752 (1993).
3. Beck-Sickinger, A. G., and G. Jung. Structure-activity relationships of neuropeptide Y analogues with respect to Y_1 and Y_2 receptors. *Biopolymers* **37:**123–142 (1995).
4. Gehlert, D. R. Subtypes of receptors for neuropeptide Y: implications for the targeting of therapeutics. *Life Sci.* **55:**551–562 (1994).
5. Grundemar, L., and R. Håkanson. Neuropeptide Y effector systems: perspectives for drug development. *Trends Pharmacol. Sci.* **15:**153–159 (1994).
6. Grundemar, L., and R. Håkanson. Multiple neuropeptide Y receptors are involved in cardiovascular regulation. Peripheral and central mechanisms. *Gen. Pharmacol.* **24:**785–796 (1993).
7. Fuhlendorff, J. U., U. Gerther, L. Aakerlund, N. L. Johansen, H. Thøgersen, S. G. Melberg, U. B. Olsen, O. Thastrup, and T. W. Schwartz. [Leu^{31},Pro^{34}]neuropeptide Y-a specific Y_1 receptor agonist. *Proc. Natl. Acad. Sci. USA* **87:**182–186 (1990).
8. Wahlestedt, C., N. Yanaihara, and R. Håkanson. Evidence for different pre- and post-junctional receptors for neuropeptide Y and related peptides. *Regul. Pept.* **13:**307–318 (1986).
9. Wahlestedt, C., S. Regunathan, and R. Håkanson. Identification of cultured cells selectively expressing Y1-, Y2-, or Y3-type receptors for neuropeptide Y/peptide YY. *Life Sci.* **50:**PL7–PL14(1992).
10. Stanley, B. G., W. Magdalin, A. Seirafi, M. M. Nguyen, and S. F. Leibowitz. Evidence for neuropeptide Y mediation of eating produced by food deprivation and for a variant of the Y1 receptor mediating this peptide's effect. *Peptides* **13:**581–587 (1992).
11. Kalra, S. P., M. G. Dube, A. Fournier, and P. S. Kalra. Structure-function analysis of stimulation of food intake by neuropeptide Y: effects of receptor agonists. *Physiol. Behav.* **50:**5–9 (1991).
12. Eva, C., K. Keinänen, H. Monyer, P. Seeburg, and R. Sprengel. Molecular cloning of a novel G protein-coupled receptor that may belong to the neuropeptide receptor family. *FEBS Lett.* **271:**80–84 (1990).
13. Herzog, H., Y. J. Hort, J. Shine, and L. A. Selbie. Molecular cloning, characterization, and localization of the human homolog to the reported bovine NPY Y3 receptor: lack of NPY binding and activation. *DNA Cell Biol.* **12:**465–471 (1993).
14. Krause, J., C. Eva, P. H. Seeburg, and R. Sprengel. Neuropeptide Y_1 subtype pharmacology of a recombinantly expressed neuropeptide receptor. *Mol. Pharmacol.* **41:**817–821 (1992).
15. Larhammar, D., A. G. Blomquist, F. Yee, E. Jazin, H. Yoo, and C. Wahlestedt. Cloning and functional expression of a human neuropeptide Y/peptide YY receptor of the Y1 subtype. *J. Biol. Chem.* **267:**10935–10938 (1992).
16. Lundell, I., A. G. Blomquist, M. Berglund, D. A. Schober, D. Johnson, R. Gadski, D. R. Gehlert, and D. Larhammar. Cloning of a human receptor of the NPY receptor family with high affinity for pancreatic polypeptide and peptide YY. *J. Biol. Chem.* **270:**29123–29128 (1995).
17. Dumont, Y., A. Fournier, S. St-Pierre, T. W. Schwartz, and R. Quirion. Differential distribution of neuropeptide Y_1 and Y_2 receptors in rat brain. *Eur. J. Pharmacol.* **191:**501–503 (1990).
18. Gehlert, D. R., S. L. Gackenheimer, and D. A. Schober. [Leu^{31}-Pro^{34}]Neuropeptide Y identifies a subtype of ^{125}I-labelled peptide YY binding sites in the rat brain. *Neurochem Int.* **21:**45–67 (1992).
19. Sheikh, S. P., M. M. T. O'Hare, O. Tortora, and T. W. Schwartz. Binding of monoiodinated neuropeptide Y to hippocampal membranes and human neuroblastoma cell lines. *J. Biol. Chem.* **264:**6648–6654 (1989).
20. Devereux, J., P. Haeberli, and O. Smithies. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* **12:**387–95 (1984).
21. Rudolf, K., W. Eberlein, W. Engel, H. A. Wieland, K. D. Willim, M. Entzeroth, W. Wienen, A. G. Beck-Sickinger, and H. N. Doods. The first highly potent and selective nonpeptide neuropeptide-Y Y-1-receptor antagonist: BIBP3226. *Eur. J. Pharmacol.* **271:**R11–R13 (1994).
22. Balasubramaniam, A., S. Sheriff, M. E. Johnson, M. Prabhakaran, Y. Huang, J. E. Fischer, and W. T. Chance. [D-Trp32]Neuropeptide Y: a competitive antagonist of NPY in rat hypothalamus. *J. Med. Chem.* **37:** 811–815 (1994).
23. Wimalawansa, S. J. Purification and biochemical characterization of neuropeptide Y_2 receptor. *J. Biol. Chem.* **270:**18523–18530 (1995).
24. Walker, P., M. Munoz, R. Martinez, and M. C. Peitsch. Acidic residues in extracellular loops of the human Y1 neuropeptide Y receptor are essential for ligand binding. *J. Biol. Chem.* **269:**2863–2869 (1994).
25. Colmers, W. F., and D. Bleakman. Effects of neuropeptide Y on the electrical properties of neurons. *Trends Neurosci.* **17:**373–379 (1994).
26. Widdowson, P. S. Quantitative receptor autoradiography demonstrates a differential distribution of neuropeptide-Y Y1 and Y2 receptor subtypes in human and rat brain. *Brain Res.* **631:**27–38 (1993).
27. Tessel, R. E., D. W. Miller, G. A. Misse, X. Dong, and M. B. Doughty. Characterization of vascular postsynaptic neuropeptide Y receptor function and regulation. 1. NPY-induced constriction in isolated rat femoral artery rings is mediated by both Y1 and Y2 receptors: evidence from benextramine protection studies. *J. Pharmacol. Exp. Ther.* **265:**172–177 (1993).
28. Lundberg, J. M., A. Hemsen, O. Larsson, A. Rudehill, A. Saria, and B. B. Fredholm. Neuropeptide Y receptor in pig spleen: binding characteristics, reduction of cyclic AMP formation and calcium antagonist inhibition of vasoconstriction. *Eur. J. Pharmacol.* **145:**21–29 (1988).
29. Rose, P. M., P. Fernandez, J. S. Lynch, S. T. Frazier, S. M. Fisher, K. Kodukula, B. Kienzle, and R. Seetha. Cloning and expression of a cDNA encoding a human type 2 neuropeptide Y receptor. *J. Biol. Chem.* **270:** 22661–22664 (1995)

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