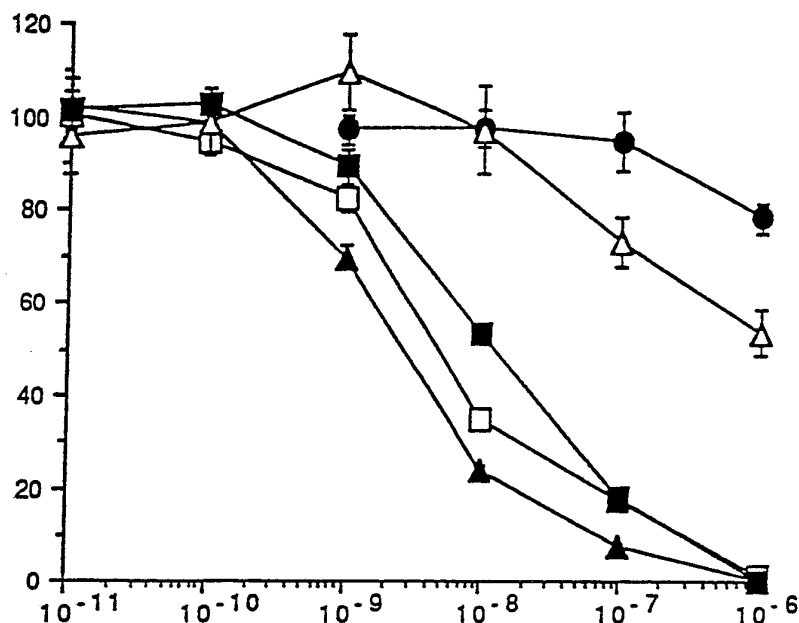




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## (54) Title: HUMAN NEUROPEPTIDE Y-Y1 RECEPTOR



## (57) Abstract

The invention provides a cDNA sequence and a genomic DNA sequence which encodes the human neuropeptide Y-Y1 receptor. These DNA sequences can be used to express the NPY-Y1 receptor in cells and can be used to screen compounds for neuropeptide Y agonist and antagonist activity.

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- 1 -

HUMAN NEUROPEPTIDE Y-Y1 RECEPTORField of the Invention

The present invention relates to cDNA and genomic DNA sequences which encode the human neuropeptide Y-Y1 receptor. In addition the present invention relates to the use of these sequences in the production of the human neuropeptide Y-Y1 receptor and related receptor subtypes using recombinant DNA technology and to methods of screening and testing compounds for neuropeptide Y (NPY) agonist or antagonist activity.

Background of the Invention

Neuropeptide Y (NPY) has a wide range of functions in the body, particularly affecting the cardiovascular system. Within the peripheral nervous system NPY is present in postganglionic sympathetic nerves, being co-localised and co-released with other neurotransmitter, including catecholamines. When used pharmacologically, NPY has been shown to have a potent vasoconstrictor activity as well as dramatically potentiating the vasoconstriction caused by many other pressor agents. Particularly high concentrations of NPY are found in the sympathetic nerves supplying the coronary, cerebral and renal vasculature and when infused into these vascular beds, NPY causes prolonged vasoconstriction that is not reversed by adrenergic blocking agents. These observations have led to the proposal that NPY is the candidate transmitter for pathological vasospasm, a major cause of morbidity and mortality when involving the coronary and cerebral vessels.

NPY also appears to be involved in interaction with the renin angiotensin system. NPY containing sympathetic nerve terminals are found on the juxta-glomerular apparatus of the renal cortex and NPY influences renin release. These data, together with the demonstration of alterations in NPY concentrations in hypertensive animal

models and the pressor response to infusion of the peptide, have resulted in implications of this peptide in hypertension.

Within the central nervous system NPY is localised  
5 predominantly within interneurons where it appears to have  
a regulatory role. It therefore has widespread and  
diverse effects including effects on memory and a possible  
role in Alzheimer's disease. NPY is the most potent known  
10 substance to cause an increase in feeding and may play a  
role in the genetic basis of Type II diabetes mellitus.  
NPY may also play a role as a regulatory agent in  
pituitary function as well as potential neuromodulatory  
function in stress responses and in reproductive function.

Specific agonists and antagonists of NPY are  
15 therefore likely to be of substantial benefit for therapy  
of a wide range of clinical disorders. As NPY possess a  
compact tertiary structure and different parts of the  
molecule are required for interaction with different  
subtypes of the receptor, the logical development of both  
20 agonists and antagonists is critically dependent upon the  
availability and knowledge of specific receptor structure.

NPY binds specifically to at least two receptors, Y1  
and Y2. (Fuhlendorff, J., et al., Proc. Natl. Acad. Sci.  
USA. 87:182-186, 1990). In addition, a third receptor  
25 subtype has been suggested (Wahlstedt, et al, Life  
Sciences 50:PL7-PL12, 1991; Michel, MC Trends in  
Pharmacol. Sci. 12:389-394, 1991). While it has been  
demonstrated that NPY receptors couple to the adenylate  
cyclase second messenger system, it remains probable that  
30 additional NPY receptor subtypes exist since there is  
evidence that phosphatidylinositol turnover, cations, and  
arachidonic acid may also function as second messengers  
for NPY. Since NPY agonists and antagonists may have  
commercial value as potential anti-hypertensive agents,  
35 cardiovascular drugs, neuronal growth factors,

- 3 -

anti-psychotics, anti-obesity and anti-diabetic agents, the ability to produce NPY receptors by recombinant DNA technology would be advantageous.

The present inventors have isolated full length cDNA clones encoding the human Y1 NPY receptor (designated Y1) from human hippocampal cDNA using DNA homology screening. The receptor sequences were identified as the human Y1 NPY receptor by expression of the cloned cDNA in mammalian cells and by measurement of specific binding to the transfected cells by a variety of NPY analogues. The receptor has also been shown to couple to both the inhibition of adenylate cyclase activity and increases in intracellular cytosolic calcium levels. In addition, the receptor has been expressed in a bacterial cells, allowing for additional drug screening methods as well as purification of the receptor protein. The DNA sequences represents a novel human receptor which may be of clinical and commercial importance.

#### Summary of the Invention

Accordingly, in a first aspect the present invention consists in a cDNA molecule encoding the human NPY-Y1 receptor, the cDNA molecule having a sequence substantial as shown in Table 1 or a functionally equivalent sequence.

In a second aspect the present invention consists in a genomic DNA molecule encoding the human NPY-Y1 receptor, the genomic DNA molecule having a sequence substantially as shown in Table 2 or a functionally equivalent sequence.

As used herein the term "functionally equivalent sequence" is intended to cover minor variation in the DNA sequence which, due to degenerancy in the DNA code, do not result in the sequence encoding a different polypeptide. Further, this term is intended to cover alterations in the DNA code which lead to changes in the encoded polypeptide, but in which such changes do not affect the biological activity of the peptide. In addition, this term is

intended to cover use of the human NPY Y1 receptor gene or gene fragments for expression in cell lines to be used in drug screening.

In a third aspect the present invention consists in a method of producing human NPY-Y1 receptors comprising culturing a cell transformed with the cDNA molecule of the first aspect of the present invention or the genomic DNA molecule of the second aspect of the present invention under conditions which allow expression of the DNA sequence and optionally recovering the human NPY-Y1 receptor.

Where the cDNA sequence is used the cells may be either mammalian cells or bacterial cells. Where the cells are mammalian cells it is presently preferred that the cells are Chinese Hamster Ovary (CHO) cells or human embryonic kidney 293 cells.

It will also be clear to persons skilled in the art that where the genomic DNA sequence is used that gene fragments could be used to obtain expression of the NPY-Y1 receptor. It is intended that the use of such gene fragments is included within the scope of the present invention.

In a further preferred embodiment the cDNA molecule is under the control of the CMV promoter when expressed in mammalian cells.

In a fourth aspect the present invention consists in a method of screening compounds for NPY agonist or antagonist activity, comprising contacting the molecule with the human NPY-Y1 receptor produced by the method of the second aspect of the present invention.

In a preferred embodiment of the present invention the NPY-Y1 receptor is present on the surface of a cell, preferably CHO or 293 cells or bacterial cells.

The cDNA and genomic DNA molecules of the present invention represent novel human receptors. These

receptors may be of interest both clinically and commercially as they are expressed in many regions of the body and a NPY affects a wide number of systems.

By using the cDNA or genomic DNA sequences of the present invention it is possible to isolate the neuropeptide Y-Y1 receptor protein in a substantially pure form.

Accordingly, in a fifth aspect the present invention consists in neuropeptide Y-Y1 receptor in a substantially pure form.

It is believed that sequence of the present invention will also enable the isolation of DNA sequences encoding other NPY-Y1 receptor subtypes including the NPY-Y2 and NPY-Y3 receptor, by using the proof in DNA homology screening of DNA libraries of interest.

In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described with reference to the following examples and drawings in which:-

Figure 1. Inhibition of porcine [ $^{125}$ I]-PYY binding with various NPY-related peptides. Increasing concentrations of human NPY [ $\square$ ], human [ $L^{31}, P^{34}$ ]NPY [ $\blacksquare$ ], porcine peptide YY [ $\blacktriangle$ ], peptide YY residues 13-36 [ $\bullet$ ], and human pancreatic polypeptide [ $\Delta$ ] were tested for their ability to inhibit the binding of [ $^{125}$ I]-PYY. Results were expressed as a percentage of the maximal specifically bound radiolabelled PYY. Standard errors of triplicate samples are shown. Untransfected CHO cells showed no specific binding of NPY.

Figure 2. The cyclic AMP response, in 293 cells transfected with human NPY Y1 receptor, to NPY ( $L^{31}, P^{34}$ ) (Y1) or porcine PYY-(13-36) is expressed as a percentage of the cyclic AMP level produced with 10 micromolar forskolin from three independent experiments.

Basal levels were  $2.5 \pm 0.13$  pmol per  $10^6$  cells (untransfected 293 cells) and  $2.26 \pm 0.06$  pmol per  $10^6$  cells (transfected 293 cells). Cyclic AMP levels stimulated by 10 micromolar forskolin were  $121.2 \pm 19.8$  pmol per  $10^6$  cells (untransfected 293 cells) and  $139 \pm 14.2$  pmol per  $10^6$  cells (transfected 293 cells).

Figure 3. Intracellular calcium levels of fura-2-loaded CHO cells transfected with the human NPY Y1 receptor cDNA were measured in response to NPY, NPY (L<sup>31</sup>,P<sup>34</sup>) (Y1), PYY, PYY 13-36, or PP. After successive addition of two of these compounds the intracellular calcium response mediated by the endogenous bombesin receptor was measured with the addition of 1 micromolar bombesin (Bom). The NPY Y1 receptor expressing cell line was stimulated with 100 nM NPY(A), peptide YY (B), NPY (L,P) (C), peptide YY cells did not respond to any of the NPY analogues, including 100 nM NPY (L<sup>31</sup>,P<sup>34</sup>) (F). Treatment of stably transfected cells overnight with pertussis toxin at 100 ng/ml abolished the response to 2.5 micromolar PYY (H), as compared with untreated cells (G), but did not affect the intracellular response to 1 micromolar bombesin. The intracellular calcium increase mediated by the NPY Y1 receptor was dependent on the concentration of NPY (L<sup>31</sup>,P<sup>34</sup>) and was maximal at 10 nM (J).

Figure 4 shows the restriction map for SacI, XhoI, BamHI and EcoRI of  $\lambda$ C clone.

Figure 5 shows the results obtained when CHO cells expressing human NPY-Y1 receptor were loaded with FURA-2 AM and stimulated with 5nM or 50nM human NPY after the addition of 40  $\mu$ g/ml hexapeptide. Increases in calcium induced by the endogenous bombesin receptor were measured by the addition of 5 nM bombesin.

Methods:

## Isolation of cDNA

Total RNA (3 $\mu$ g) from rat brain was used as a template to synthesize random primed single-stranded  
5 cDNAs. These cDNAs were used in a polymerase chain reaction (PCR) together with the oligonucleotide primers R1 (CTG GTG CTG CAG TAT TTT GGC CCA CTC TGT) and R2 (AAT GTC TCA GAG AAT TCT CCA TTT CTG GCC) 30 pmol each) which correspond to position 672-584 and 48-78 in the rat cDNA  
10 clone FC5R, respectively. PCR condition: 30 cycles at 95 for 1 min, 63 C for 2 min and 72 C for 1 min. The reaction product was digested with EcoR I and Pst I, gel purified and subcloned for sequencing into the Bluescript vector (Stratagene) to show authenticity.

15 Two lambda cDNA libraries derived from human fetal brain (Clonetec) and human adult hippocampus (Stratagene) (9.10<sup>5</sup> pfu each) were screened with the rat cDNA as a probe under following hybridization conditions: 5 x SSPE, 0.1% SDS and 5x Denhardt at 60 C for 16 hours. The  
20 filters were washed twice with 2 x SSC and 0.1% SDS at 60 C for 15 min. Three strongly hybridizing clones were isolated and the cDNA inserts were subcloned for sequencing into Bluescript vectors. The largest cDNA (2.5 kb) contains an open reading frame for 384 amino acids  
25 encoding the human NPY receptor subtype Y<sub>1</sub> (Table 1). The two other clones (F5 and F13) are truncated versions of the same cDNA with 100% identity in the overlapping region (position 664-1555 and 670-1925) respectively.

The mammalian expression construct pN-H(3-4) was made  
30 by subcloning a PCR fragment containing only the coding region of the NPY Y<sub>1</sub> receptor into the pcDNA NEO vector. The construct is under the control of the CMV promoter and contains the neomycin gene for selection.

The expression construct pN-H3 was transfected into  
35 the mammalian cell line CHO K1 using a modified calcium

TABLE 1/1  
Human Neuropeptide Y Y1 receptor cDNA

```

* * * * *
ATTGTT CAGTTC AAGGGAATGAAGAATTCAGAATAATTTTGGTAAATGGATTCCAATATCGGGAATAAGA 70
* * * * *
* * * * *
ATAAGCTGAACAGTTGACCTGCTTTGAAGAAACATACTGTCCATTGTCTAAAATAATCTATAACAACCA 140
* * * * *
* * * * *
AACCAATCAAAATGAATTCAACATTATTTTCCCAGGTTGAAAATCATTTCAGTCCACTCTAATTTCTCAGA 210
M N S T L F S Q V E N H S V H S N F S E
* * * * *
* * * * *
GAAGAATGCCAGCTTCTGGCTTTTGAAGAAATGATGATTGTTCATCTGCCCTTGGCCATGATATTTACCTTA 280
K N A Q L L A F E N D D C H L P L A M I F T L
* * * * *
* * * * *
GCTCTTGCTTATGGAGCTGTGATCATTCTTGGTGTCTCTGGAAACCTGGCCTTGATCATAATCATCTTGA 350
A L A Y G A V I I L G V S G N L A L I I I I L
* * * * *
* * * * *
AACAAAAGGAGATGAGAAATGTTACCAACATCCTGATTGTGAACCTTTCCTTCTCAGACTTGCTTGTTC 420
K Q K E M R N V T N I L I V N L S F S D L L V A
* * * * *
* * * * *
CATCATGTGTCTCCCCTTTACATTTGTCTACACATTAATGGACCACTGGGTCTTTGGTGAGGCGATGTGT 490
I M C L P F T F V Y T L M D H W V F G E A M C
* * * * *
* * * * *
AAGTTGAATCCTTTTGTGCAATGTGTTTCAATCACTGTGTCCATTTTCTCTCTGTTCTCATTGCTGTGG 560
K L N P F V Q C V S I T V S I F S L V L I A V
* * * * *
* * * * *
AACGACATCAGCTGATAATCAACCCTCGAGGGTGGAGACCAATAATAGACATGCTTATGTAGGTATTGC 630
E R H Q L I I N P R G W R P N N R H A Y V G I A
* * * * *
* * * * *
TGTGATTTGGGTCCTTGCTGTGGCTTCTTCTTGCCTTTCCTGATCTACCAAGTAATGACTGATGAGCCG 700
V I W V L A V A S S L P F L I Y Q V M T D E P
* * * * *
* * * * *
TTCCAAAATGTAACACTTGATGCGTACAAAGACAAATACGTGTGCTTTGATCAATTTCCATCGGACTCTC 770
F Q N V T L D A Y K D K Y V C F D Q F P S D S
* * * * *
* * * * *
ATAGGTTGTCTTATACCCTCTCCTCTTGGTGCTGCAGTATTTTGGTCCACTTTGTTTTATATTTATTG 840
H R L S Y T T L L L V L Q Y F G P L C F I F I C
* * * * *
* * * * *
CTACTTCAAGATATATATACGCCTAAAAAGGAGAAACAACATGATGGACAAGATGAGAGACAATAAGTAC 910
Y F K I Y I R L K R R N N M M D K M R D N K Y
* * * * *
* * * * *
AGGTCCAGTGAAACCAAAGAATCAATATCATGCTGCTCCTCATTGTGGTAGCATTGTCAGTCTGCTGGC 980
R S S E T K R I N I M L L S I V V A F A V C W
* * * * *
* * * * *
TCCCTCTTACCATCTTTAACACTGTGTTTGGATTGGAATCATCAGATCATTGCTACCTGCAACCACAATCT 1050
L P L T I F N T V F D W N H Q I I A T C N H N L

```

TABLE 1/2

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* * * * * 1120
GTTATTCCTGCTCTGCCACCTCACAGCAATGATATCCACTTGTGTCAACCCCATATTTTATGGGTTCCCTG
L F L L C H L T A M I S T C V N P I F Y G F L
* * * * * 1190
AACAAAACTTCCAGAGAGACTTGCAGTTCTTCTCAACTTTTGTGATTTCCGGTCTCGGGATGATGATT
N K N F Q R D L Q F F F N F C D F R S R D D D
* * * * * 1260
ATGAAACAATAGCCATGTCCACGATGCACACAGATGTTTCCAAAACCTTCTTTGAAGCAAGCAAGCCAGT
Y E T I A M S T M H T D V S K T S L K Q A S P V
* * * * * 1330
CGCATTTAAAAAATCAACAACAATGATGATAATGAAAAAATCTGAACTACTTATAGCCTATGGTCCCG
A F K K I N N N D D N E K I *
* * * * * 1400
GATGACATCTGTTTAAAAACAAGCACAACCTGCAACATACTTTGATTACCTGTTCTCCAAGGAATGGGG
* * * * * 1470
TTGAAATCATTGAAAATGACTAAGATTTTCTTGTCTTGCTTTTTACTGCTTTTGTGTAGTTGTCATAA
* * * * * 1540
TTACATTTGGAACAAAAGGTGTGGGCTTTGGGGTCTTCTGAAATAGTTTTGACCAGACATCTTTGAAGT
* * * * * 1610
GCTTTTTGTGAATTTATGCATATAATAAAGACTTTTATACTGTACTTATTGGAATGAAATTTCTTTAA
* * * * * 1680
AGTATTACGATNNNCTGACTTCAGAAGTACCTGCCATCCAATACGGTCATTAGATTGGGTCATCTTGATT
* * * * * 1750
AGATTAGATTAGATTAGATTGTCAACAGATTGGGCCATCCTTACTTTATGATAGGCATCATTTTAGTGTG
* * * * * 1820
TTACAATAGTAACAGTATGCAAAGCAGCATTGAGGAGCCGAAAGATAGTCTTGAAGTCATTCAGAAGTG
* * * * * 1890
GTTTGAGGTTTCTGTTTTTTGGTGGTTTTTGTGTTTTTTTTTTTTTTTTCACCTTAAGGGAGGCTTTCAT
* * * * * 1960
TTCTCCCGACTGATTGTCACTTAAATCAAATTTAAAAATGAATAAAAAGACATACTTCTCAGCTGCAA
* * * * * 2030
ATATTATGGAGAATTGGGCACCCACAGGAATGAAGAGAGAAAGCAGCTCCCCAACTTCAAACCATTTTG
* * * * * 2100
GTACCTGACAACAAGAGCATTTTAGAGTAATTAATTTAATAAAGTAAATTAGTATTGCTGCAAATAGCTA
* * * * * 2170
AATTATATTTATTTGAATTGATGGTCAAGAGATTTTCCATTTTTTTTACAGACTGTTTCAGTGTGTTGTCAA
* * * * * 2240
GCTTCTGGTCTAATATGTACTCGAAAGACTTTCCGCTTACAATTTGTAGAAACACAAATATCGTTTTCCA
* * * * * 2310
TACAGCAGTGCCTATATAGTGACTGATTTTAACTTTCAATGTCCATCTTTCAAAGGAAGTAACACCAAGG
* * * * * 2380
TACAATGTTAAAGGAATATTCACCTTACCTAGCAGGGAAAAATACACAAAACCTGCAGATACTTCATATA

```

TABLE 1/3

2450

\* \* \* \* \*  
GCCCATTTTAACTTGTATAAACTGTGTGACTTGTGGCGTCTTATAAATAATGCACTGTAAAGATTACTGA  
2520

\* \* \* \* \*  
ATAGTTGTGTCATGTTAATGTGCCTAATTCATGTATCTTGTAATCATGATTGAGCCTCAGAATCATTG  
2590

\* \* \* \* \*  
GAGAACTATATTTTAAAGAACAAGACATACTTCAATGTATTATACAGATAAAGTATTACATGTGTTGA

\* \* \* \* \*  
TTTTAAAAGGGCGGACATTTTATTTAAAATCAAGG

-11-

phosphate transfection method. Stably transfected cells were selected with neomycin and tested for the ability to bind NPY/PYY peptide analogues. Transfected cells ( $1 \times 10^6$ ) were incubated in 0.5ml assay buffer (50mM Tris-HCl  
5 pH 7.4, 2mM  $\text{CaCl}_2$ , 5mM  $\text{KCl}$ , 120mM  $\text{NaCl}$ , 1mM  $\text{MgCl}_2$ , and 0.1% bovine serum albumin) in the presence of 0.05nM [ $^{125}\text{I}$ ]-labelled peptide YY(NEN) and either  $10^{-8}\text{M}$  porcine peptide YY(PYY 13-36), or the Y1 receptor-selective NPY(Leu31,Pro34)[peptides were obtained  
10 from Auspep, Australia]. Peptide analogues were also tested on human neuroblastoma SK-N-MC cells ( $1.6 \times 10^6$ ), which express only the Y1 receptor subtype, and on rat brain membrane preparations (approximately 100 $\mu\text{g}$ ) which express predominantly Y2 receptors. Untransfected  
15 CHO K1 cells ( $1 \times 10^6$ ) were also tested and showed no specific binding to the peptide analogues. Cells were incubated with the radiolabelled peptide and unlabelled competitors for one hour at room temperature and pelleted in a microcentrifuge for 4 minutes. Pellets were counted  
20 for one minute in a gamma counter. Binding of the Y1 receptor-selective agonist confirmed the identity of this clone as the NPY-Y1 receptor.

The expressed NPY Y1 receptor was assessed for its ability to bind NPY and related analogs (methods detailed  
25 in human NPY Y1 receptor patent application) (Figure 1). The receptor has been successfully used to show specific binding to a Y1 receptor-specific agonist NPY ( $\text{L}^{31}, \text{P}^{34}$ ) and to not bind to a Y2 receptor-specific agonist peptide YY 13-36. These results reflect the in vivo effects of  
30 these agonists.

In the present invention, the Y1 receptor expressed in these cell lines has been shown to couple to inhibition of adenylate cyclase in 293 cells (Figure 2) and the increase in cytosolic calcium levels in the CHO cells  
35 (Figure 3). These are significant and novel findings and

SUBSTITUTE SHEET
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-12-

of use in the screening of agonists and antagonists by function.

To measure intracellular cyclic AMP levels, cyclic AMP was assayed in whole cells treated for 15 min. at 5 37°C with 100 micromolar isobutylmethylxanthine (IBMX; Sigma). Transfected cells ( $1 \times 10^6$  / 0.5 ml reaction) were incubated with 10 micromolar forskolin and various concentrations of NPY and related peptides. Reactions were terminated with the addition of HCl to 0.1M, 10 incubation at room temperature for 15 min., neutralisation and sample dilution in 50 mM sodium acetate, pH 6.2. Cyclic AMP was quantitated by using a radioimmunoassay (Dupont/NEN).

To measure levels of intracellular calcium, 15 transfected cells were suspended in loading medium (modified RPMI 1640 medium/10 mM HEPES/1% newborn calf serum) and incubated in a spinner flask at 37°C for 2.5 hour at  $1 \times 10^6$  cells per ml. Cells were then treated with 1 micromolar Fura-2 acetoxymethyl ester (fura-2 AM; 20 Molecular Probes) for 30 min. at 37°C, washed twice with loading medium, and resuspended at  $5 \times 10^6$  cells/ml. Immediately before fluorescence spectroscopy, cells were recovered by centrifugation at 1000 rpm and resuspended at  $1 \times 10^6$  cells/ml in a modified Krebs buffer (135 mM NaCl/ 25 4.7 mM KCl/1.2 mM MgSO<sub>4</sub>/1.2 mM KH<sub>2</sub>PO<sub>4</sub>/5 mM NaHCO<sub>3</sub>/ 1 mM CaCl<sub>2</sub>/2.8 mM glucose/10 mM HEPES, pH 7.4) containing sulfinpyrazone. Bombesin was purchased from Sigma and Auspep. Fluorescence recordings were made on a Hitachi fluorescence spectrometer (F4010) at 340 nm 30 (excitation) and 505 nm (emission) over 10 min. with slit widths of 5 nm and response time of 2 seconds. Intracellular calcium was quantitated by using equations described by Grynkiewicz, et al., J. Bio. Chem. 260:3440-3450, 1985.

**SUBSTITUTE SHEET**

-13-

Plasmid Construction

Plasmid pMalp (Promega), which carries the male gene under the control of the tac promoter, was cut with EcoRI and HindII restriction enzymes simultaneously. A 2.1kb  
5 EcoRI/HindIII cDNA fragment containing the whole coding region for the human NPY Y1 receptor was cloned into the pMalp vector to generate plasmid pHz59. The plasmid pHz59 was cut with StuI and EcoRI restriction enzymes  
10 simultaneously and the 5' overhang of the EcoRI site was filled in with Klenow enzyme and the plasmid was religated to generate the fusion construct pHz60. The recombinant plasmid (pHz60) was transfected into the bacterial strain TB1.

Preparation and Fractionation of E.coli Membranes

15 Bacteria were grown at 37°C in L broth containing 50µg/ml of ampicillin, up to an A600 value of 0.5-0.7. Derepression of the tac promoter with 1mM isopropylb-D-thiogalactopyranoside (IPTG) for 2 hours at  
20 37 C let to the production of a 86 kD male/NPY Y1 receptor fusion-protein. Cells, from a 11 culture, were harvested and washed at 4°C with 11 10mM Hepes pH 7.5 and the pellet stored over night at -20°C. All the subsequent steps were carried out on ice. The bacterial pellet was  
25 suspended in 28 ml 10mM Hepes pH 7.5 containing 20% (by mass) sucrose, DNase I at 30 µg/ml, and RNase A at 30 µg/ml, and the following protease inhibitors: 1mM phenylmethylsulfonylfluoride, leupeptin at 5 µg/ml, and pepstatin at 7 µg/ml. The suspension was sonicated three  
30 times for 3 min. Unbroken cells were removed by centrifugation at 5000g for 10 min. and 2 ml 0.1 M EDTA (pH 7.5) was added to the supernatant. This supernatant was layered on a sucrose gradient consisting of 3ml 60% (mass/vol.) sucrose, 6 ml 42.5% (mass/vol.) sucrose, and  
35 15 ml 25% (mass/vol.) sucrose (in 10 mM Hepes pH7.5 containing 5 mM EDTA) and centrifuged at 100000g for 16h

**SUBSTITUTE SHEET**

-14-

at 4°C in a Beckmann SW41 rotor. Two major bands were visible at the interface of the sucrose layers, corresponding to the enriched preparations of the outer and inner membrane. Fractions were collected from the  
5 gradient and aliquots used to measure binding activity for <sup>125</sup>I NPY, in the binding assay described.

#### Isolation of the Human NPY Y1 Receptor Gene

A human genomic DNA library constructed in lambda GEM  
11 phage vector (Clontec) was screened with a  
10 <sup>32</sup>P-labelled cloned NPY Y1 receptor cDNA (nucleotides 14 to 2327) isolated from human hippocampal cDNA. Two positive clones were obtained from 7.5x10<sup>5</sup> bacteriophage plaques. Clone kC contained an insert of approximately 14 kb and clone kD contained an insert of approximately  
15 11kb. The insert in kD was subsequently shown to be completely contained within the longer clone. The restriction map for Sac I, Xho I, BamH I and EcoR I of the kC clone is shown in Fig. 4 and sequence set out in Table 2. The insert of this clone was digested with  
20 different enzymes and the fragments subcloned into the Bluescript SK vector for sequencing. The exon sequence of the NPY Y1 receptor gene is identical to that of the human hippocampal cDNA described above. The human NPY Y1  
25 receptor gene consists of 3 exons. This is in contrast to many of the other G protein coupled receptor genes, which are intronless. The overall sequence of the gene consists of approximately 10kb (Fig. 4). The first 57 nucleotides of the 5' untranslated sequence of the human hippocampal NPY Y1 receptor mRNA are separated by a 6kb intron from  
30 the second exon. The second intron (97bp), containing an in frame stop codon, is located exactly after the proposed fifth transmembrane domain at nucleotide 908 corresponding to the cDNA sequence (Table 2). Introns in several other G-coupled receptor genes (human substance K, human  
35 rhodopsin), tend to be positioned the same way, shortly

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TABLE 2/1

\* \* \* \* \*

AACGTACTCGTGTACATTCTATTTTTTCTTCATAATGTTTCAGTACTGTAGTACTAATCACCGAGAAAAT

\* \* \* \* \*

TGCATTGACTCTTTTTCGACCACCAGGAAATATTCAGCTCATGGTTCCTCCCAAAAAAACTAAAAAGCAG

\* \* \* \* \*

CTAAGCGCTGGGAACAAATCTGACTTATTGCATTTTCTCAGTGGGCCAAAGAAAGGAGGGCCGATTGACT

\* \* \* \* \*

GCTTTGACTTTTTAAAGGTCTTCTCTTTGTTCACTTATAAAGTGAGGAAAACAAATTCTCGGCACTGGCG

\* \* \* \* \*

TGAGAGTTGAGCGTCACAAAAGAAAGCAAAAGAAAATATTAGTGCCATTATTGTGGCGAATTTTCATGTTT

\* \* \* \* \*

CCCAGCGAGCCCTTTGATTCTGGTTTGGGCTGGCGCTCGAGCTCTCCAGCCGGGTATGACTTCGGCCAC

\* \* \* \* \*

AAGATGGCACTGACCTGCAAAACAAAGAAAAGCACAGTGGCACCGACTTTTTCAAGCCTCGGGAAACTGCC

\* \* \* \* \*

CTGCCTTCCCCGGAGTCGAGGACTGTGGGGATTAGGGCTTCTTTCCCTGCGCGGGAGGTCTGTGTGCGA

\* \* \* \* \*

ATAATGTGTGGCTTCTGTTGGATTGCTTTTCTTTCCAAAATTCCTAGGCAATGCTTCCCCGAGGTGTGCA

\* \* \* \* \*

CCTTTGTGAGGTGTTTGTGGGGTTGGGGGAGCTTCAGGCGCTACTCGCGGGACGACGTCA<sup>CRE</sup>CGTGATCCGG

\* \* \* \* \*

GATGAGGTGGAGTTCGGCTTTAAGGAGGCGTCTCTTCTAGCTTCAATCAATCTTTAGGATCTGAGCAGGA

\* \* \* \* \*

GAAATAC<sup>TATA</sup>CAGCGGATCTTCCCACCTGCTCCCTTCCATTCCCACCCTTCTTCTTTAATAAGCAGGAGC

\* \* \* \* \*

GAAAAAGACAAATTCCAAAGAGG<sup>+57</sup>GTAAGTTGCGAGTTTATGCCTTTCCAGAGACTTCTGCGAAATCTCTC

-----

ATTGACAAGGTGAAGGATGAGAGGGGAAGAAAACGATGCGAGTGTCCGAAACTGGCTCTGGGGGACCAA

-----

GGTGGGGTCTCCAGTGCAGGCAGGTGCGAGGTTGCTCCAATCACCGGACCGTTTCGCGGGCTCCTGCCGA

-----

AGGGTATGGGGACGACGCGGTGAAAGGAGAGGGTACCCTACGCGGAGTTCGGGCTTTCCCCCACCTGC

-----

TCCCGGGGAATTTCTGGATGGGGATCCAAGTTTCTTTACCCCGGTTCTCTTTAAAGGCCGAGGCACT

-----

GGGAAGGCGCCCGAGTTTCGCTATCCCACACCCGCTCATTTTCTTACGTGTCTTGAGCTGGCTGGAGGC

-----

ACTGGCTCTGGCCGCACCGGAGTTTTCGCGGAGTAACTGGCTGGGATGAGCCGGAGAAGGTGGGCTTGC

-----

ACGTCGCCCAGCGTGCACGGGTCCGCGGCAGGTGTCGGGCTGGGAGCTGGCGGAGGTGGGAGTAGCCCT

-----

CGGCGCGGACCCAGCGCGCAAGCCCACCCCACTTGGGGTTCCGCGCTTCTCGGCTGCAATCGAGCCG

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TABLE 2/2

\* \* \* \* \*

CGCACCGCAGTACCGGGCGCTTCGAGTGTGGCGCTGCGCCCCGATAGACACCCGAAGCTTTTAATCATCG

---

\* \* \* \* \*

GAGTCTAATCAGGGTCTCTTTAGCTCTTTCTTTAGAAAAGTAATGATGGAACGTGGCTGG-----

----- 6 kb -----

\* \* \* \* \*

-----TATTCCTTACTAACTTATAGACCATTATGTTTACTAAGACTTGTTCTGTAAGCAAGTCC

\* \* \* \* \*

AATTAAAAATTTTATTTTTCTTTTATTTCAGTATGTTTTCACCATTCTGCTATTTTAGAAAGATGTTTAC

\* \* \* \* \*

AAGATTACATTTGTTTATTTATTTCAGTGTTCCTTAAAGAGTTCTGTGAGTCAGAAGTCATTTTG

\* \* \* \* \* +58 \*

ACTGCCCTCAATAAAAATTAGTAATGCAATTGGTCATTTCTCTTTACAGTTGTTTCAGTTCAAGGGAATG

\* \* \* \* \*

AAGAATTCAGAATAATTTGGTAAATGGATTCCAATATCGGGATAAGAATAAGCTGAACAGTTGACCTG

\* \* \* \* \*

CTTTGAAGAAACATACTGTCCATTTGTCTAAAATAATCTATAACAACCAAACCAATCAAAATGAATTCAA  
M N S

\* \* \* \* \*

CATTATTTTCCCAGGTTGAAAATCATTTCAGTCCACTCTAATTTCTCAGAGAAGAATGCCAGCTTCTGGC  
T L F S Q V E N H S V H S N F S E K N A Q L L A

\* \* \* \* \*

TTTTGAAAATGATGATTGTCATCTGCCCTTGGCCATGATATTTACCTTAGCTCTTGCTTATGGAGCTGTG  
F E N D D C H L P L A M I F T L A L A Y G A V

\* \* \* \* \*

ATCATTCTTGGTGTCTCTGGAAACCTGGCCTTGATCATAATCATCTTGAAACAAAAGGAGATGAGAAATG  
I I L G V S G N L A L I I I I L K Q K E M R N

\* \* \* \* \*

TTACCAACATCCTGATTGTGAACCTTTCCTTCTCAGACTTGCTTGTTGCCATCATGTGTCTCCCCTTAC  
V T N I L I V N L S F S D L L V A I M C L P F T

\* \* \* \* \*

ATTTGTCTACACATTAATGGACCCTGGGTCTTTGGTGAGGCGATGTGTAAGTTGAATCCTTTTGTGCAA  
F V Y T L M D H W V F G E A M C K L N P F V Q

\* \* \* \* \*

TGTGTTTCAATCACTGTGTCCATTTCTCTCTGGTTCCTCATTTGCTGTGGAACGACATCAGCTGATAATCA  
C V S I T V S I F S L V L I A V E R H Q L I I

\* \* \* \* \*

ACCTCGAGGGTGGAGACCAAATAATAGACATGCTTATGTAGGTATTGCTGTGATTGGGTCTTGGTGT  
N P R G W R P N N R H A Y V G I A V I W V L A V

\* \* \* \* \*

GGCTTCTTCTTTGCCTTTCCTGATCTACCAAGTAATGACTGATGAGCCGTTCCAAAATGTAACACTTGAT  
A S S L P F L I Y Q V M T D E P F Q N V T L D

\* \* \* \* \*

GCGTACAAAGACAAATACGTGTGCTTTGATCAATTTCCATCGGACTCTCATAGGTTGTCTTATAACACTC  
A Y K D K Y V C F D Q F P S D S H R L S Y T T

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TABLE 2/3

```

*           *           *           *           *           *           *           *           *           *
TCCTCTTGGTGCTGCAGTATTTTGGTCCACTTTGTTTTATATTTATTTGCTACTTCAAGCTAAGAAAAC
L L L V L Q Y F G P L C F I F I C Y F K
*           *           *           *           *           *           *           *           *           *
TTTTTCTATCATTTCATTTTACCTTCTTTACACAGAATTCCTCATCAAATGAGTGTTCATTAA
*           *           *           *           *           *           *           *           *           *
CTTTTTCTTCCATAGATATATATACGCCTAAAAGGAGAAACAACATGATGGACAAGATGAGAGACAAT
I Y I R L K R R N N M M D K M R D N
*           *           *           *           *           *           *           *           *           *
AAGTACAGGTCCAGTGAACCAAAGAATCAATATCATGCTGCTCTCCATTGTGGTAGCATTTCAGCTCT
K Y R S S E T K R I N I M L L S I V V A F A V
*           *           *           *           *           *           *           *           *           *
GCTGGCTCCCTCTTACCATCTTTAACACTGTGTTGATTGGAATCATCAGATCATTGCTACCTGCAACCA
C W L P L T I F N T V F D W N H Q I I A T C N H
*           *           *           *           *           *           *           *           *           *
CAATCTGTTATTCCTGCTCTGCCACCTCACAGCAATGATATCCACTTGTGTCAACCCCATATTTATGGG
N L L F L L C H L T A M I S T C V N P I F Y G
*           *           *           *           *           *           *           *           *           *
TTCCTGAACAAAACTTCCAGAGAGACTTGCAGTTCTTCTCAACTTTTGTGATTTCCGGTCTCGGGATG
F L N K N F Q R D L Q F F F N F C D F R S R D
*           *           *           *           *           *           *           *           *           *
ATGATTATGAAACAATAGCCATGTCCAGATGCACACAGATGTTTCCAAACTTCTTTGAAGCAAGCAAG
D D Y E T I A M S T M H T D V S K T S L K Q A S
*           *           *           *           *           *           *           *           *           *
CCCAGTCGCATTTAAAAAATCAACAACAATGATGATAATGAAAAATCTGAACTACTTATAGCCTATG
P V A F K K I N N N D D N E K I
*           *           *           *           *           *           *           *           *           *
GTCCCGGATGACATCTGTTAAAAACAAGCACACCTGCAACATACTTTGATTACCTGTTCTCCAAGGA
*           *           *           *           *           *           *           *           *           *
ATGGGGTTGAAATCATTGAAATGACTAAGATTTTCTTGCTTGCTTTTACTGCTTTTGTGTAGTTG
*           *           *           *           *           *           *           *           *           *
TCATAATTACATTTGGAACAAAAGGTGTGGGCTTTGGGGTCTTCTGAAATAGTTTGGACCAGACATCTT
*           *           *           *           *           *           *           *           *           *
TGAAGTGCTTTTTGTGAATTTATGCATATAATATAAAGACTTTTATACTGTACTTATTGGAATGAAATTT
*           *           *           *           *           *           *           *           *           *
CTTTAAAGTATTACGATNNCTGACTTCAGAAGTACCTGCCATCCAATACGGTCATTAGATTGGGTCATC
*           *           *           *           *           *           *           *           *           *
TTGATTAGATTAGATTAGATTAGATTGTCAACAGATTGGGCCATCCTTACTTTATGATAGGCATCATTTT
*           *           *           *           *           *           *           *           *           *
AGTGTGTTACAATAGTAACAGTATGCAAAGCAGCATTGAGGAGCCGAAAGATAGTCTTGAAGTCATTCA
*           *           *           *           *           *           *           *           *           *
GAAGTGTTGAGGTTTCTGTTTTTGGTGGTTTTTGTGTTTTTTTTTTTTTTTACCTTAAGGGAGGC
*           *           *           *           *           *           *           *           *           *
TTTCATTCCTCCCGACTGATTGTCACTTAAATCAAATTTAAAAATGAATAAAAAGACATACTTCTCAG

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TABLE 2/4

\* \* \* \* \*

CTGCAAATATTATGGAGAATTGGGCACCCACAGGAATGAAGAGAGAAAGCAGCTCCCCAACTTCAAACC

\* \* \* \* \*

ATTTTGGTACCTGACAACAAGAGCATTTTAGAGTAATTAATTTAATAAAGTAAATTAGTATTGCTGCAA

\* \* \* \* \*

TAGCTAAATTATATTTATTTGAATTGATGGTCAAGAGATTTCCATTTTTTTTACAGACTGTTTCAAGTGT

\* \* \* \* \*

TGTC AAGCTTCTGGTCTAATATGTACTCGAAAGACTTTCCGCTTACAATTTGTAGAAACACAAATATCGT

\* \* \* \* \*

TTTCCATACAGCAGTGCCTATATAGTACTGATTTTAACTTAATGTCCATCTTTCAAAGGAAGTAACA

\* \* \* \* \*

CCAAGGTACAATGTTAAAGGAATATTCACCTTTACCTAGCAGGGAAAAATACACAAAACTGCAGATACTT

\* \* \* \* \*

CATATAGCCCATTTTAACTTGTATAAACTGTGTGACTTGTGGCGTCTTATAAATAATGCACTGTAAAGAT

\* \* \* \* \*

TACTGAATAGTTGTGTCATGTTAATGTGCCTAATTTCAATGATCTTGTAAATCATGATTGAGCCTCAGAAT

\* \* \* \* \*

CATTTGGAGAACTATATTTTAAAGAACAAGACATACTTCAATGTATTATACAGATAAAGTATTACATGT

\* \* \* \* \*

GTTTGATTTTTAAAAGGGCGGACATTTTATTAAAATCAAGG

after or in front of a transmembrane domain. This organisation is also consistent with suggestions that the following third cytoplasmic loop of the receptor forms a specific domain involved in determination of the specificity of coupling to different G proteins. The nucleotide sequences of the two introns adjoining the splice junctions (Table 2) are consistent with the recognised consensus sequence GT/AG.

#### Putative regulatory sequence elements

10 Identification of the transcriptional initiation site was carried out with primer extension, using a 21mer primer corresponding to nucleotides 38 to 18. The primer when extended on mRNA derived from the NPY Y1 receptor specific expressing human neuroblastoma cell line SK-N-MC, 15 revealed a transcription start site at 210 nucleotides upstream from the initiation start codon. This position is also identical with a type 1 cap site (CCATTC) and is accompanied 35bp upstream by a TATA box-like motif (AAATAC), a typical CAAT box (TCAATCT) 60bp upstream, a 20 cAMP response element (CGACGTCA) 124 bp upstream and a AP2 recognition site (GCGAGCCC) 451bp upstream (Table 2). No other typical transcription factor binding sites are found in the + orientation in this region. A potential polyadenylation site (ATTAAA) was found at position 2670 25 of the cDNA sequence. The approximate 2.7 kb sequence of the cDNA is consistent with the size of the NPY Y1 receptor mRNA demonstrated in Northern analysis of placental and kidney mRNA.

#### Characterisation of a Pst I Polymorphism in the Human NPY

##### 30 Y1 Receptor Gene

Southern blot analysis was performed using the <sup>32</sup>P-labelled cDNA fragment (nucleotides 14 to 2327) as a probe. The restriction patterns obtained with BamH I, EcoR I and Sac I correspond exactly to the fragments found 35 in the human genomic clones. However, the pattern for the

-20-

restriction enzyme Pst I shows an additional 5.1 kb band, which suggests the presence of a polymorphic site within the first intron of the gene. Southern blot analysis of genomic DNA samples from 69 normal individuals confirmed  
5 this suggestion and demonstrated that the allele frequency for the Pst I polymorphism in this population is 54%:46%. (Table 3). Sequence analysis revealed a single point mutation in the recognition site for the restriction enzyme Pst I, changing the first cytidine to a thymidine.  
10 The polymorphism could be used to assess variations in the population which may represent a possible marker for hypertension.

#### Genomic Library Screening

A human peripheral blood genomic DNA library  
15 (Clontec) was screened with a <sup>32</sup>P-labelled 2.3kb fragment (nucleotides 14 to 2327) of the human NPY Y1 receptor cDNA. Bacteriophage DNA was transferred to Hybond N<sup>+</sup> filters (Amersham) and hybridised with the probe in a solution containing 6xSSC, 5xDenhardt's and  
20 0.1% SDS at 65°C for 16 h. Filters were washed twice 15 min in 2xSSC/0.1%SDS at 65°C followed by a 15 min wash in 0.1xSSC/0.1% SDS and exposed to X-ray film (Kodak, X-Omat) using an intensifying screen at -70°C for 16h. Positive plaques were purified and DNA was isolated using  
25 a standard lysate procedure. cDNA was digested with EcoR I, Hind III, BamH I, Sac I, Xho I, or combinations of these enzymes to generate subsequently ordered subclones in the Bluescript SK vector (Stratagene) covering the entire region of the NPY Y1 receptor gene.

#### 30 Nucleotide Sequence Determination

Supercoiled plasmid DNA was alkaline-denatured and sequenced by the dideoxy chain termination method using T7  
polymerase (Promega). Oligonucleotide primers were initially from the flanking region of the vector and  
35 internal to the cDNA sequence. Additional primers were

<b>SUBSTITUTE SHEET</b>
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synthesised based on intron sequence obtained.

TABLE 3: NPY Y1 Receptor Gene Pst I Polymorphism

5	Genotypes			Allele Frequency	
	3.8/3.8	3.8/5.1	5.1/5.1	3.8	5.1
Samples (69)	22	30	17	74	64
10 Frequency	31.9%	43.4%	24.6%	53.6%	46.4%

NOY Agonist and Antagonist Sequeasing

The mammalian cells transfected with the human NPY Y1 receptor cDNA were used to identify a peptide antagonist of NPY function.

Using synthetic peptide chemistry, a hexapeptide amide SALRHY-NH<sub>2</sub> (Ser-Ala-Leu-Arg-His-Tyr amide), which corresponds to residues 22-27 of the NPY molecule portion of the amphiphatic helix in NPY was synthesised in an ABI Peptide Synthesiser Model 430A. T-boc chemistry was used. HF cleavage was used to release peptide from the solid support. Peptide was subjected to HPLC (Ion Exchange and Reverse Phase) purifications. The peptide was subjected to sequence and amino acid analysis for sequence integrity. In a series of experiments not described here in anaesthetized rats significant inhibition of the NPY-evoked pressor response and a decrease in resting blood pressure levels 60 minutes after administration of SALRHY-NH<sub>2</sub> was observed. The inhibitory effect of SALRHY-NH<sub>2</sub> is confined to the postsynaptic or Y1 receptor as no significant inhibitory effects are seen on attenuation of cardiac vagal action, a Y2 receptor mediated function.

-22-

The hexapeptide was tested in vitro for its ability to inhibit NPY effects on mammalian cell lines transfected with the human NPY Y1 receptor cDNA. The ability of the hexapeptide to block the NPY-induced increase in intracellular calcium correlated well with its in vivo ability to block NPY Y1 receptor-mediated increases in blood pressure.

CHO cells expressing the human NPY Y1 receptor were loaded with FURA-2 AM as described, and stimulated with 5 nM or 50 nM human NPY (Auspep) after the addition of 401g/ml SALRHY-NH2 hexapeptide. Increases in calcium induced by the endogenous bombesin receptor were measured by the addition of 5nM bombesin. The results are shown in Figure 5.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

**SUBSTITUTE SHEET**

-23-

## CLAIMS:-

1. A cDNA molecule encoding the human NPY-Y1 receptor, the cDNA molecule having a sequence substantial as shown in Table 1 or a functionally equivalent sequence.
- 5 2. A genomic DNA molecule encoding the human NPY-Y1 receptor, the genomic DNA molecule having a sequence substantially as shown in Table 2 or a functionally equivalent sequence.
3. A method of producing human NPY-Y1 receptors  
10 comprising culturing a cell transformed with the cDNA molecule as claimed in claim 1 or genomic DNA molecule as claimed in claim 2 under conditions which allow expression of the DNA sequence and optionally recovering the human NPY-Y1 receptor.
- 15 4. A method as claimed in claim 3 in which the method comprises culturing a bacterial cell transformed with the cDNA molecules as claimed in claim 1.
5. A method as claimed in claim 3 in which the cell is a mammalian cell.
- 20 6. A method as claimed in claim 5 in which the cells are Chinese Hamster Ovary cells or human embryonic kidney 293 cells.
7. A method as claimed in claim 5 or 6 in which the cell is transformed with a fragment of the genomic DNA molecule  
25 as claimed in claim 2, the fragment including the coding regions for the NPY-Y1 receptor.
8. A method as claimed in any one of claims 3 to 7 in which the cDNA molecule is under the control of the CMV promoter.
- 30 9. A method of screening compounds for NPY agonist or antagonist activity, comprising contacting the molecule with the human NPY-Y1 receptor produced by the method as claimed in any one of claims 3 to 8.
10. A method as claimed in claim 9 in which the NPY1  
35 receptor is present on the surface of a cell.

SUBSTITUTE SHEET
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-24-

11. A method as claimed in claim 10 in which the cell is a mammalian or bacterial cell.
12. A method as claimed in claim 11 in which the cell is Chinese Hamster Ovary cell or human embryonic kidney 293  
5 cells.
13. Neuropeptide Y-Y1 receptor in a substantially pure form.

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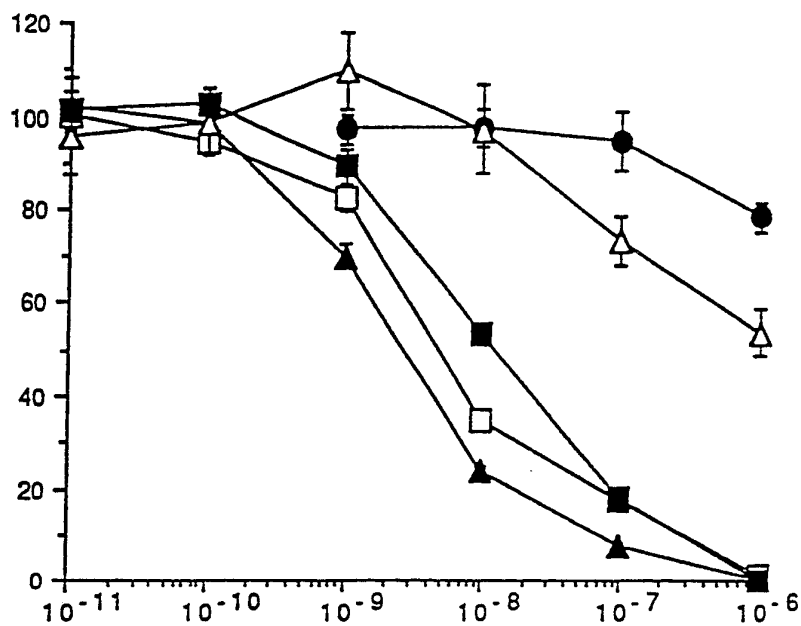


Fig. 1

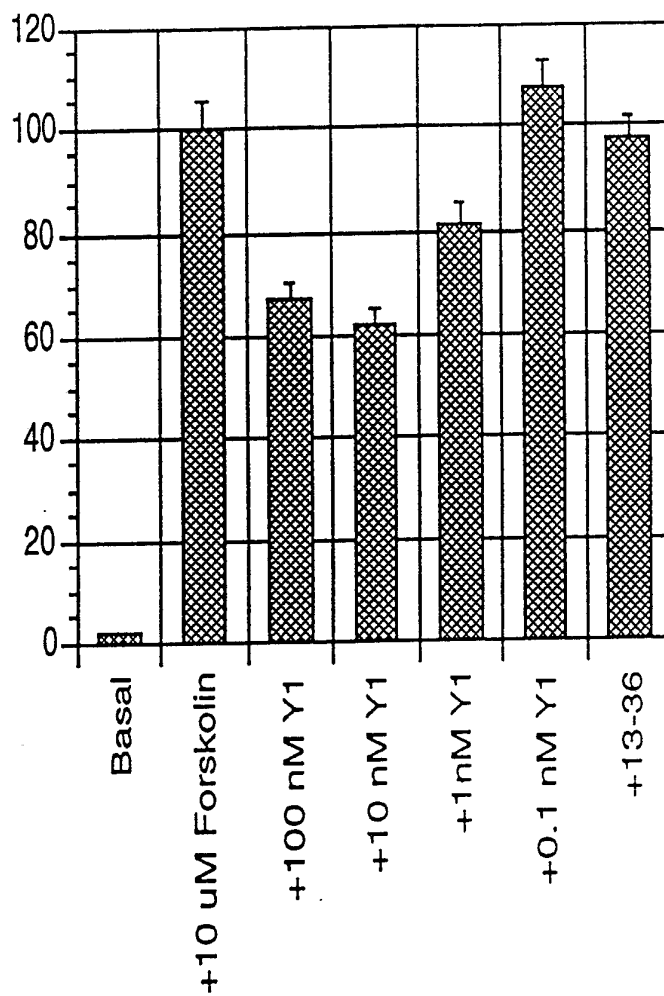


Fig.2

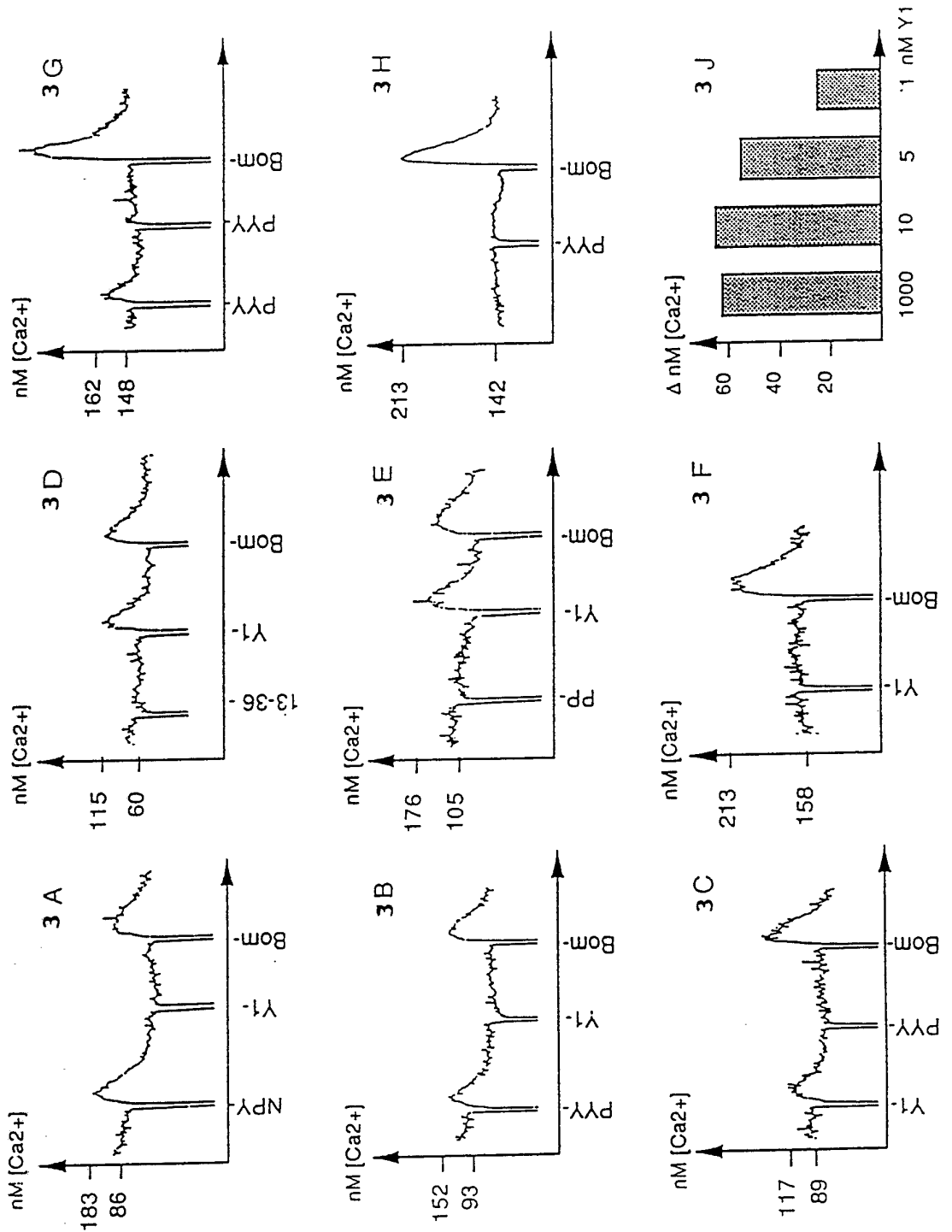


Figure 3.

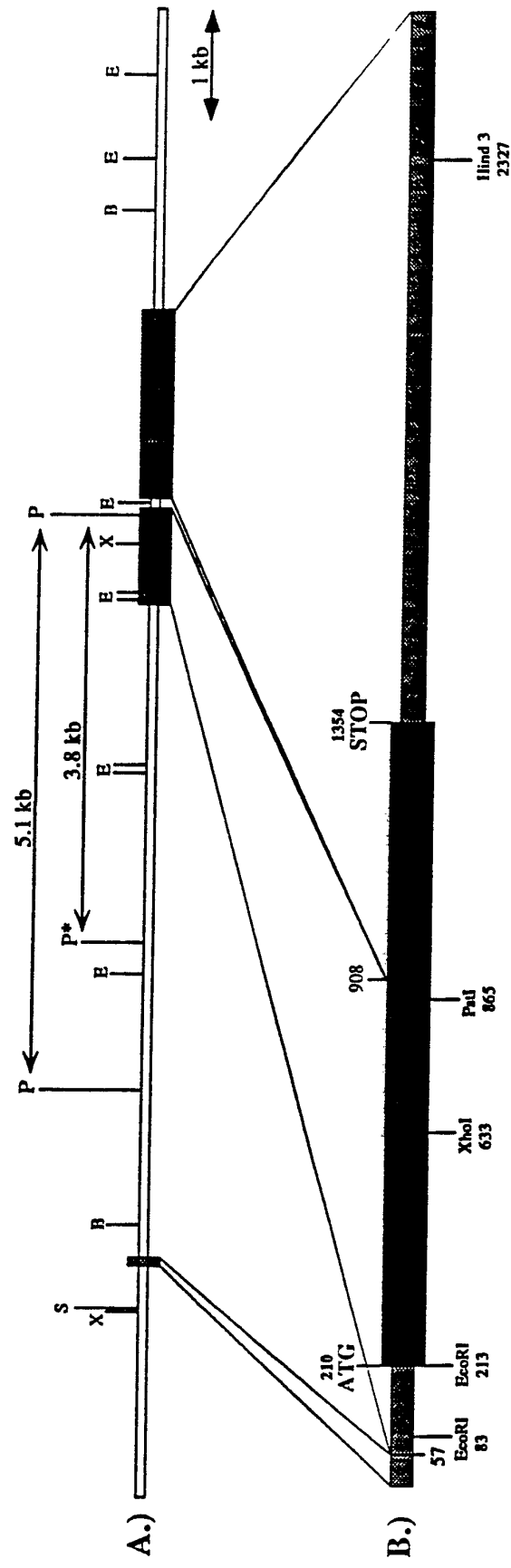


FIG. 4

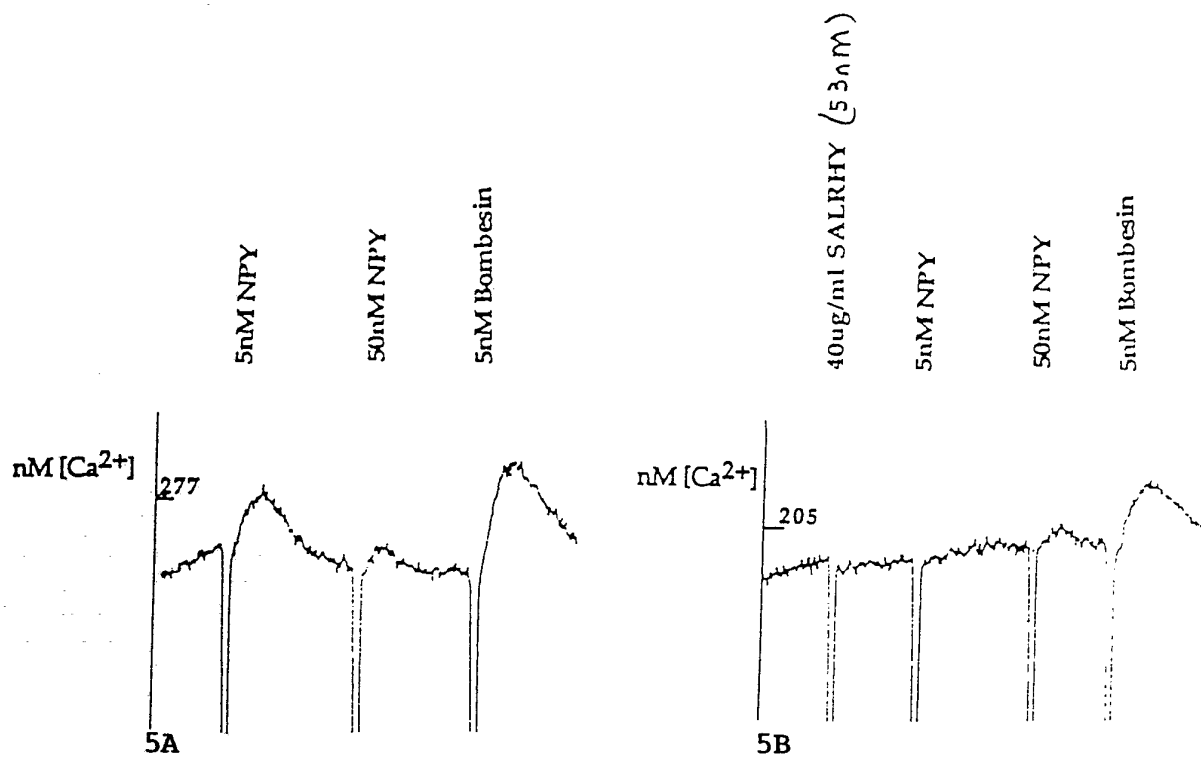



FIG. 5

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> Int. Cl. <sup>5</sup> C12N 15/12 C07K 13/00  According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>  Minimum documentation searched (classification system followed by classification symbols) WPAT, CHEMICAL ABSTRACTS DATABASES KEYWORDS : NEUROPEPTIDE, Y, Y <sub>1</sub> , RECEPTOR  Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched AU : C12N 15/12 C07K 13/00; BIOTECHNOLOGY ABSTRACTS  Electronic data base consulted during the international search (name of data base, and where practicable, search terms used) WPAT, CHEMICAL ABSTRACTS, BIOTECHNOLOGY ABSTRACTS KEY WORDS AS ABOVE		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	SHEIKH S P et al "Y <sub>1</sub> and Y <sub>2</sub> receptors for neuropeptide Y" FEBS LETTERS, published March 1989 volume 245 pp 209-214.	1-13
A	FR,A, 2628750 (INSTITUT PASTEUR) 22 September 1989 (22.09.89) See page 2 and claim 1.	1
A	EP,A, 355793 (MERRELL DOW PHARMACEUTICALS INC) 28 February 1990 (28.02.90) See pages 2 and 3 and examples.	1
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents :		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier document but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	
Date of the actual completion of the international search 15 February 1993 (15.02.93)	Date of mailing of the international search report 19 FEB 1993 (19.02.93)	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA  Facsimile No. 06 2853929	Authorized officer  J H CHAN  Telephone No. (06) 2832340	

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
A	EP,A, 355794 (MERRELL DOW PHARMACEUTICALS INC) 28 February 1990 (28.02.90) See pages 2 and 3 and examples.	1
A	Feth et al "G-Protein coupling and signalling of Y <sub>1</sub> -like neuropeptide Y receptors in SK-N-MC cells" Archives of Pharmacology (1991) Volume 344 pages 1-7.	1
A	Sheikh S.P. et al "Binding of Monoiodinated Neuropeptide Y to Hippocampal Membranes and Human Neuroblastoma Cell lines" Journal of Biological Chemistry issued 25 April 1989, vol 264 pages 6648-6654.	1
A	Aakerlund L et al "Y <sub>1</sub> receptors for neuropeptide Y are coupled to mobilization of intracellular calcium and inhibition of adenylate cyclase" FEBS LETTERS published January 1990, volume 260 pages 73-78.	1