



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : C07H 21/02, 21/04, C07K 14/705, C12N 5/10, 15/12, 15/63, C12P 21/02, G01N 33/566, 33/567	A1	(11) International Publication Number: <b>WO 96/23809</b> (43) International Publication Date: 8 August 1996 (08.08.96)
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(21) International Application Number: PCT/US96/01444  
(22) International Filing Date: 30 January 1996 (30.01.96)  
(30) Priority Data:  
383,746 3 February 1995 (03.02.95) US  
415,818 3 April 1995 (03.04.95) US

(60) Parent Applications or Grants  
(63) Related by Continuation  
US 383,746 (CIP)  
Filed on 3 February 1995 (03.02.95)  
US 415,818 (CIP)  
Filed on 3 April 1995 (03.04.95)

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(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

**Published**

*With international search report.*

(54) Title: NEUROPEPTIDE Y RECEPTOR

**(57) Abstract**

A novel mammalian neuropeptide Y receptor and method of making the receptor are provided. The invention includes DNA encoding the receptor, assays employing the receptor, cells expressing the receptor, antibodies which bind specifically to the receptor, RNA encoded by the DNA sequence or its complementary sequence, and single-stranded DNA with a sequence complementary to the RNA which encodes the receptor. The receptor and assays employing the receptor are useful for identifying compounds which bind to the receptor, including specific modulators of the receptor. Such compounds are useful for treating a variety of disease conditions, including obesity, diabetes, anxiety, hypertension, cocaine withdrawal, congestive heart failure, memory enhancement, cardiac and cerebral vasospasm, pheochromocytoma and ganglioneuroblastoma, and Huntington's, Alzheimer's and Parkinson's diseases.

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- 1 -

TITLE OF THE INVENTION  
NEUROPEPTIDE Y RECEPTOR

BACKGROUND OF THE INVENTION

5           This application is a continuation-in-part of copending application U.S. Serial No. 08/415,818, filed April 3, 1995, which is a continuation-in-part of copending application U.S. Serial No. 08/383,746, filed February 3, 1995, the contents of both of which are hereby incorporated by reference.

10           Neuropeptide Y (NPY) is a 36 residue, amidated polypeptide. It is anatomically co-distributed and co-released with norepinephrine in and from sympathetic postganglionic neurons ([1], [2], [3], [4], [5], [6]). Stimulation of the sympathetic nervous system under physiological circumstances such as exercise ([7], [8]) or exposure  
15 to the cold ([9], [10]) promotes an elevation of both norepinephrine and NPY.

          NPY is believed to act in the regulation of appetite control ([11], [12]) and vascular smooth muscle tone ([13], [14]) as well as regulation of blood pressure ([6], [15], [16], [17]). NPY also decreases  
20 cardiac contractility ([18], [19], [20], [21], [22]). Congestive heart failure and cardiogenic shock are associated with probable releases of NPY into the blood ([23], [24], [25]). Regulation of NPY levels may be beneficial to these disease states [26].

          At the cellular level, neuropeptide Y binds to a G-protein  
25 coupled receptor ([27], [28], [29], [30]). Neuropeptide Y is involved in regulating eating behavior and is an extremely potent orexigenic agent ([11], [12], [31]). When administered intracerebroventricularly or injected into the hypothalamic paraventricular nucleus (PVN) it elicits eating in satiated rats ([32], [33], [34]) and intraventricular injection of  
30 antisera to NPY decreases eating ([11], [31]). It has been shown to stimulate appetite in a variety of species and at different stages of development ([12]). Other effects on energy metabolism include decreased thermogenesis, body temperature and uncoupling protein, and increased white fat storage and lipoprotein lipase activity ([9], [35], [36],  
35 [37], [38], [39]). NPY levels in the PVN increase upon fasting ([40],

- 2 -

[41], [42], [43], [44]), before a scheduled meal ([31], [36], [40]), and in both streptozotocin-induced and spontaneous diabetes ([36], [45], [46], [47], [48], [49]). Also, NPY levels are increased in genetically obese and hyperphagic Zucker rats ([36], [50], [51]). Thus, a specific  
5 centrally acting antagonist for the appropriate NPY receptor subtype may be therapeutically useful for treating obesity and diabetes. Other disorders which might be targeted therapeutically include anxiety, hypertension, cocaine withdrawal, congestive heart failure, memory enhancement, cardiac and cerebral vasospasm, pheochromocytoma and  
10 ganglioneuroblastoma, and Huntington's, Alzheimer's and Parkinson's diseases ([26], [52]).

At least four receptor subtypes of the NPY family have been proposed based on pharmacological and physiological properties. The Y1 receptor is stimulated by NPY or PYY (peptide YY) and  
15 appears to be the major vascular receptor ([16], [53], [54], [55]). The Y2 receptor is stimulated by C-terminal fragments of NPY or PYY and is abundantly expressed both centrally and peripherally ([55], [56], [57], [58]). A third receptor (Y3) is exclusively responsive to NPY and is likely present in adrenal medulla, heart, and brain stem ([27], [59]). In  
20 addition, other subtypes of this receptor family are known to exist, based on pharmacological and physiological characterization ([60], [61], [62], [63]). The feeding behavior is stimulated potently by NPY, NPY<sub>2-36</sub> and the Y1 agonist [Leu<sup>31</sup>, Pro<sup>34</sup>]NPY, but is not stimulated by the Y2 agonist NPY<sub>13-36</sub> ([11], [64], [65], [66]). This pharmacology is not  
25 characteristic of the defined Y1, Y2 or Y3 receptors and can thus be attributed to a unique receptor, termed "atypical Y1" ([11], [65], [66]), that is responsible for evoking the feeding response. In addition, data indicate the existence of additional members of this receptor family including one subtype specific for peptide PP ([62], [63]), one with  
30 affinity for short C-terminal fragments of NPY which induce hypotension when administered systemically ([15], [17], [30], [67], [68]), and one associated with binding of NPY and PYY to brain sigma and phencyclidine binding sites ([61]).

- 3 -

The Y1 receptor has been cloned and shown to be a G-protein coupled receptor ([53], [69], [70]). Recently, the Y2 receptor has been cloned ([71], [72]). In addition a peptide PP preferring receptor, termed PPI ([73]) or Y4 [(74)], has been cloned. Until the  
5 invention described herein of a novel NPY receptor, other NPY receptors had not been cloned.

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#### SUMMARY OF THE INVENTION

The present invention relates to an isolated DNA sequence encoding a novel neuropeptide Y receptor, hereinafter referred to as the neuropeptide Y Y<sub>x</sub> receptor. The DNA sequence encoding the NPY Y<sub>x</sub> receptor has a sequence selected from a sequence as shown in Figure 3, a sequence of substantial homology to the sequence shown in Figure 3, a

- 11 -

fragment of the sequence shown in Figure 3, a fragment of the sequence of substantial homology to the sequence shown in Figure 3, a sequence as shown in Figure 5, a sequence of substantial homology to the sequence shown in Figure 5, a fragment of the sequence shown in  
5 Figure 5 or a fragment of the sequence of substantial homology to the sequence shown in Figure 5.

In one embodiment of the invention is the isolated DNA sequence wherein the sequence is selected from a sequence as shown in Figure 3, a sequence of substantial homology to the sequence shown in  
10 Figure 3, a fragment of the sequence shown in Figure 3 or a fragment of the sequence of substantial homology to the sequence shown in Figure 3.

In a class of the invention is the DNA sequence which encodes a murine NPY Y<sub>x</sub> receptor.

15 In a subclass of the invention is the DNA sequence having a sequence selected from the sequence shown in Figure 3, the sequence of substantial homology to the sequence shown in Figure 3 or the fragment of the sequence shown in Figure 3 comprising bases 822 to 1934.

In a second embodiment of the invention is the isolated  
20 DNA sequence wherein the DNA sequence has a sequence selected from a sequence as shown in Figure 5, a sequence of substantial homology to the sequence shown in Figure 5, a fragment of the sequence shown in Figure 5 or a fragment of the sequence of substantial homology to the sequence shown in Figure 5.

25 In a second class of the invention is the DNA sequence which encodes a human NPY Y<sub>x</sub> receptor.

In a second subclass is the DNA sequence having a sequence selected from the sequence shown in Figure 5, the sequence of substantial homology to the sequence shown in Figure 5 or the fragment  
30 of the sequence shown in Figure 5 comprising bases 182 to 1291.

Illustrative of the invention is an expression vector containing any of the DNA sequences described above.

An illustration of the invention is a cell transformed by the expression vector.

- 12 -

Exemplifying the invention is a method of producing the neuropeptide Y Y<sub>x</sub> receptor, comprising culturing the cell under conditions which allow the production of the neuropeptide Y Y<sub>x</sub> receptor and optionally recovering the neuropeptide Y Y<sub>x</sub> receptor. An  
5 example of the invention is a neuropeptide Y Y<sub>x</sub> receptor produced by this process. Preferably, the neuropeptide Y Y<sub>x</sub> receptor produced by this process is characterized by a pharmacological binding profile with affinities of PYY ≅ NPY ≅ [Leu<sup>31</sup>Pro<sup>34</sup>]NPY ≅ NPY(2-36) > NPY(13-36). More specifically exemplifying the invention is the method  
10 wherein the cell is a mammalian cell; preferably, a COS-7 cell.

Further illustrating the invention is a neuropeptide Y Y<sub>x</sub> receptor, or a functional derivative thereof, which is characterized by a pharmacological binding profile with affinities of PYY ≅ NPY ≅ [Leu<sup>31</sup>Pro<sup>34</sup>]NPY ≅ NPY(2-36) > NPY(13-36), in substantially pure  
15 form.

More particularly illustrating the invention is an antibody immunologically reactive with the NPY Y<sub>x</sub> receptor.

Another illustration of the invention is an isolated RNA encoded by any of the DNA sequences described above or their  
20 complementary sequences.

Since a single-stranded DNA with a sequence complementary to the sequence of RNA which encodes the NPY Y<sub>x</sub> receptor could be used to modulate the expression of the receptor, another example of the invention is isolated DNA containing this  
25 complementary sequence or a fragment thereof.

Further exemplifying the invention is a neuropeptide Y Y<sub>x</sub> receptor in substantially pure form comprising an amino acid sequence selected from a sequence as shown in Figure 3 (SEQ. I.D. NO. 6), a sequence as shown in Figure 6 (SEQ. I.D. NO. 12), or a functional  
30 derivative thereof. Preferably, the neuropeptide Y Y<sub>x</sub> receptor has an amino acid sequence as shown in Figure 3 (SEQ. I.D. NO. 6) or a functional derivative thereof. More preferably, the neuropeptide Y Y<sub>x</sub> receptor has an amino acid sequence as shown in Figure 6 (SEQ. I.D. NO. 12) or a functional derivative thereof.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1. Schematic diagram of FRAMES analysis using the Genetics Computing Group software (Madison, WI) identifying a 371 amino acid open reading frame (aa ORF).

5 Figure 2. Schematic diagram of hydrophobicity analysis using PEPLOT (Genetics Computing Group software (Madison, WI)) indicating the open reading frame (ORF) has 7 transmembrane spanning domains characteristic of G-protein coupled receptor.

10 Figure 3 shows the genomic DNA sequence and deduced amino acid sequence of the murine NPY Yx receptor. The sequence disclosure of Fig. 3 is represented as SEQ. I.D. NO. 5 and SEQ. I.D. NO. 6.

15 Figure 4 shows a comparative alignment determined by BESTFIT (Genetics Computing Group software (Madison, WI)) of the mouse NPY Y1 receptor and the mouse NPY Yx receptor, described herein. The seven transmembrane spanning domains are indicated as are three putative N-linked glycosylation sites (indicated by a \*). The sequence disclosures of the mouse NPY Y1 receptor and mouse NPY Yx receptor of Fig. 4 are represented as SEQ. I.D. NO. 7 and SEQ. I.D. NO. 6, respectively.

20 Figure 5 shows the DNA sequence of the human NPY Yx receptor. The sequence disclosure of Fig. 5 is represented as SEQ. I.D. NO. 11.

25 Figure 6 shows the amino acid sequence of the human NPY Yx receptor. The sequence disclosure of Fig. 6 is represented as SEQ. I.D. NO. 12.

### DETAILED DESCRIPTION OF THE INVENTION

Neuropeptide Y receptors belong to a class of receptors known as "G-protein coupled receptors." The term "G-protein coupled receptor" refers to any receptor protein that mediates its endogenous  
5 signal transduction through activation of one or more guanine nucleotide binding regulatory proteins (G-proteins). These receptors share common structural features, including seven hydrophobic transmembrane domains. G-protein coupled receptors include receptors that bind to small biogenic amines, including but not limited to beta-  
10 adrenergic receptors ( $\beta$ AR), alpha-adrenergic receptors ( $\alpha$ AR) and muscarinic receptors, as well as receptors whose endogenous ligands are peptides, such as neurokinin, neuropeptide Y and glucagon receptors. Examples of  $\beta$ AR include beta-1, beta-2, and beta-3 adrenergic  
receptors.

15 G-protein coupled receptors are cell surface proteins that mediate the responses of a cell to a variety of environmental signals. Upon binding an agonist, the receptor interacts with one or more specific G-proteins, which in turn regulate the activities of specific effector proteins. By this means, activation of G-protein coupled  
20 receptors amplifies the effects of the environmental signal and initiates a cascade of intracellular events that ultimately leads to defined cellular responses. G-protein coupled receptors function as a complex information processing network within the plasma membrane of the cell, acting to coordinate a cell's response to multiple environmental  
25 signals.

G-protein coupled receptors consist of seven hydrophobic domains connecting eight hydrophilic domains. The hydrophobicity or hydrophilicity of the domains may be determined by standard  
hydropathy profiles, such as Kyte-Doolittle analysis (Kyte, J. and  
30 Doolittle, R.J.F. *J. Mol. Biol.* 157: 105 (1982)). The receptors are thought to be oriented in the plasma membrane of the cell such that the N-terminus of the receptor faces the extracellular space and the C-terminus of the receptor faces the cytoplasm, so that each of the hydrophobic domains crosses the plasma membrane. The receptors

- 15 -

have been modeled and the putative boundaries of the extracellular, transmembrane and intracellular domains are generally agreed (for a review, see Baldwin, EMBO J. 12:1693, 1993). In general, the transmembrane domains are comprised of stretches of 20-25 amino acids in which most of the amino acid residues have hydrophobic side chains (including cysteine, methionine, phenylalanine, tyrosine, tryptophan, proline, glycine, alanine, valine, leucine, isoleucine), whereas the intracellular and extracellular loops are defined by contiguous stretches of several amino acids that have hydrophilic or polar side chains (including aspartate, glutamate, asparagine, glutamine, serine, threonine, histidine, lysine, and arginine). Polar amino acids, especially uncharged ones (such as serine, threonine, asparagine, and glutamine) are found in both transmembrane and extramembrane regions.

The present invention pertains to a novel mammalian neuropeptide Y receptor subtype (i.e., NPY Y<sub>x</sub>), particularly exemplified by the murine and human neuropeptide Y Y<sub>x</sub> receptors described in detail herein. A method of making the NPY Y<sub>x</sub> receptor is also provided. The invention includes DNA encoding the NPY Y<sub>x</sub> receptor, the NPY Y<sub>x</sub> receptor, assays employing the NPY Y<sub>x</sub> receptor, cells expressing the NPY Y<sub>x</sub> receptor, antibodies which bind specifically to the NPY Y<sub>x</sub> receptor, RNA encoded by the DNA sequence or its complementary sequence, and single-stranded DNA with a sequence complementary to the RNA which encodes the NPY Y<sub>x</sub> receptor. The NPY Y<sub>x</sub> receptor and assays employing the NPY Y<sub>x</sub> receptor are useful for identifying compounds which bind to the receptor, including specific modulators of the receptor. Modulators, as described herein, include but are not limited to agonists, antagonists, suppressors and inducers. Such modulators may be DNA, RNA, peptides, proteins, or non-proteinaceous organic molecules. Modulators identified by the processes described herein are useful as therapeutic agents. Thus, compounds identified using the mammalian NPY Y<sub>x</sub> receptor are useful for treating one or more disease conditions, including obesity, diabetes, anxiety, hypertension, cocaine withdrawal,

- 16 -

congestive heart failure, memory enhancement, cardiac and cerebral vasospasm, pheochromocytoma and ganglioneuroblastoma, and Huntington's, Alzheimer's and Parkinson's diseases.

5 The receptor may include genetic variants, both natural and induced. Induced receptors may be derived by a variety of methods, including but not limited to, site-directed mutagenesis. Techniques for nucleic acid and protein manipulation are well-known in the art and are described generally in Methods in Enzymology and in Sambrook et al.,  
10 Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory (1989).

It is known that there is a substantial amount of redundancy in the various codons which code for specific amino acids. Therefore, this invention is also directed to those DNA sequences which contain alternative codons which code for the  
15 eventual translation of the identical amino acid. For purposes of this specification, a sequence bearing one or more replaced codons will be defined as a degenerate variation. Also included within the scope of this invention are mutations either in the DNA sequence or the  
20 translated protein which do not substantially alter the ultimate functional properties of the expressed protein. For example, substitution of valine for leucine, arginine for lysine, or asparagine for glutamine may not cause a change in functionality of the polypeptide.

It is known that DNA sequences coding for a peptide may  
25 be altered so as to code for a peptide having properties that are different than those of the naturally-occurring peptide. Methods of altering the DNA sequences include, but are not limited to site directed mutagenesis. Examples of altered properties include but are not limited to changes in the affinity of an enzyme for a substrate or a receptor for a ligand.

30 A fragment of a DNA sequence, as used herein, refers to any polynucleotide subset of the DNA sequence or its complementary sequence. Also included within the scope of the present invention are fragments of DNA sequence capable of producing functional NPY Yx protein. An example of one such fragment capable of expressing the

- 17 -

NPY Y<sub>x</sub> receptor protein comprises bases 822 to 1934 of the DNA sequence shown in Figure 3. Another example of a fragment capable of expressing the NPY Y<sub>x</sub> receptor protein comprises bases 182 to 1291 of the DNA sequence shown in Figure 5.

5           As used herein, a "functional derivative" of a receptor is a compound that possesses a biological activity (either functional or structural) that is substantially similar to the biological activity of the receptor. The term "functional derivative" is intended to include the "fragments," "variants," "degenerate variants," "analogs" and  
10 "homologues" or to "chemical derivatives" of the receptor. The term "fragment" is meant to refer to any polypeptide subset of receptor. The term "variant" is meant to refer to a molecule substantially similar in structure and function to either the entire receptor molecule or to a fragment thereof. A molecule is  
15 "substantially similar" to a receptor if both molecules have substantially similar structures or if both molecules possess similar biological activity. Therefore, if the two molecules possess substantially similar activity, they are considered to be variants even if the structure of one of the molecules is not found in the other or  
20 even if the two amino acid sequences are not identical.

The term "analog" refers to a molecule substantially similar in function to either the entire receptor molecule or to a fragment thereof.

25           The term "ligand," as used herein, refers to a molecule which binds to the receptor; the term "ligand" includes both agonists and antagonists.

"Substantial homology" or "substantial similarity," when referring to nucleic acids means that the segments or their complementary strands, when optimally aligned and compared, are  
30 identical with appropriate nucleotide insertions or deletions, in at least about 30% of the nucleotides, usually >40% of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize to a strand or its complement under standard conditions. Standard hybridization conditions are described in Sambrook, J., et al.,

- 18 -

supra. Thus, the terms "substantial homology" and "substantial similarity" are intended to cover minor variation in the DNA sequence which, due to degeneracy in the DNA code, do not result in the sequence encoding a different polypeptide; further these terms are  
5 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.

The nucleic acids claimed herein may be present in whole cells or in cell lysates or in a partially purified or substantially purified  
10 form. When referring to nucleic acids, the terms "isolated" and "substantially pure" are synonymous. A nucleic acid is considered to be isolated and/or substantially purified when it is purified away from environmental contaminants. Thus, a nucleic acid sequence isolated  
15 from cells is considered to be isolated and/or substantially purified when purified from cellular components by standard methods while a chemically synthesized nucleic acid sequence is considered to be substantially purified when purified from its chemical precursors. The  
20 term "substantially pure" is used relative to nucleic acids, proteins or peptides with which the nucleic acids of the instant invention are associated in nature, and are not intended to exclude compositions in which the nucleic acid of the invention is admixed with nonproteinous pharmaceutical carriers or vehicles.

Nucleic acid compositions of this invention may be derived from genomic DNA, cDNA, or RNA, prepared by synthesis or by a  
25 combination of techniques.

The natural or synthetic nucleic acids encoding the G-protein coupled receptor of the present invention may be incorporated into expression vectors. Usually the expression vectors incorporating the receptor will be suitable for replication in a host. Examples of  
30 acceptable hosts include, but are not limited to, prokaryotic and eukaryotic cells.

The phrase "recombinant expression system" as used herein means a substantially homogenous culture of suitable host organisms that stably carry a recombinant expression vector. Examples

- 19 -

of suitable hosts include, but are not limited to, bacteria, yeast, fungi, insect cells, plant cells and mammalian cells. Generally, cells of the expression system are the progeny of a single ancestral transformed cell.

5           The cloned receptor DNA obtained through the methods described herein may be recombinantly expressed by molecular cloning into an expression vector containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce recombinant  
10 receptor. Techniques for such manipulations are fully described in Sambrook, J., *et al.*, *supra*, and are well known in the art.

          Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned copies of genes and the translation of their mRNAs in an appropriate host. Such vectors  
15 can be used to express eukaryotic genes in a variety of hosts such as bacteria, bluegreen algae, plant cells, insect cells, fungal cells and animal cells.

          Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast or bacteria-animal cells or  
20 bacteria-fungi or bacteria-invertebrate cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a  
25 DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses.

30           A variety of mammalian expression vectors may be used to express recombinant receptor in mammalian cells. Commercially available mammalian expression vectors which may be suitable for recombinant receptor expression, include but are not limited to, pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene),

- 20 -

pSG5 (Stratagene), pCI-neo (Promega), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and  $\lambda$ ZD35  
5 (ATCC 37565).

A variety of bacterial expression vectors may be used to express recombinant receptor in bacterial cells. Commercially available bacterial expression vectors which may be suitable for recombinant receptor expression include, but are not limited to  
10 pGEM-3ZF (Promega), pET11a (Novagen), lambda gt11 (Invitrogen), pcDNAII (Invitrogen), pKK223-3 (Pharmacia).

A variety of fungal cell expression vectors may be used to express recombinant receptor in fungal cells. Commercially available fungal cell expression vectors which may be suitable for  
15 recombinant receptor expression include but are not limited to pYES2 (Invitrogen), *Pichia* expression vector (Invitrogen).

A variety of insect cell expression vectors may be used to express recombinant receptor in insect cells. Commercially available insect cell expression vectors which may be suitable for  
20 recombinant expression of receptor include but are not limited to pBlue Bac III (Invitrogen).

An expression vector containing DNA encoding receptor may be used for expression of receptor in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic,  
25 including but not limited to bacteria such as E. coli, fungal cells such as yeast, mammalian cells including but not limited to cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Cell lines derived from mammalian species which may be  
30 suitable and which are commercially available, include but are not limited to, L cells L-M(TK<sup>-</sup>) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3

- 21 -

(ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

The expression vector may be introduced into host cells via any one of a number of techniques including but not limited to  
5 transformation, transfection, lipofection, protoplast fusion, and electroporation. The expression vector-containing cells are clonally propagated and analyzed to determine whether they produce receptor protein. Identification of receptor expressing host cell clones may be done by several means, including but not limited to immunological  
10 reactivity with anti-receptor antibodies or specific ligand binding.

Expression of receptor DNA may also be performed using *in vitro* produced synthetic mRNA or native mRNA. Synthetic mRNA or mRNA isolated from receptor producing cells can be efficiently translated in various cell-free systems, including but not  
15 limited to wheat germ extracts and reticulocyte extracts, as well as efficiently translated in cell based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being preferred.

The term "substantial homology", when referring to  
20 polypeptides, indicates that the polypeptide or protein in question exhibits at least about 30% homology with the naturally occurring protein in question, usually at least about 40% homology.

The receptor may be expressed in an appropriate host cell and used to discover compounds that affect the receptor. Preferably,  
25 the receptor is expressed in a mammalian cell line, including but not limited to, COS-7, CHO or L cells, or an insect cell line, including but not limited to, Sf9 or Sf21, and may be used to discover ligands that bind to the receptor and alter or stimulate its function. The receptor may also be produced in bacterial, fungal or yeast expression systems.

30 The expression of the receptor may be detected by use of a radiolabeled ligand specific for the receptor. For example, for the  $\beta_2$  adrenergic receptor, such a ligand may be  $^{125}\text{I}$ -iodocyanopindolol ( $^{125}\text{I}$ -CYP). For the NPY receptor, such a ligand may be  $^{125}\text{I}$ -NPY or  $^{125}\text{I}$ -Peptide YY (PYY).

- 22 -

The specificity of binding of compounds showing affinity for the receptor is shown by measuring the affinity of the compounds for cells transfected with the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for  
5 compounds that inhibit the binding of radiolabeled ligand to these cells provides a rational way for rapid selection of compounds with high affinity for the receptor. These compounds identified by the above assays may be agonists or antagonists of the receptor and may be peptides, proteins, or non-proteinaceous organic molecules.  
10 Alternatively, functional assays of the receptor may be used to screen for compounds which affect the activity of the receptor. Such functional assays range from ex vivo muscle contraction assays to assays which determine second messenger levels in cells expressing the receptor. The second messenger assays include but are not limited to  
15 assays to measure cyclic AMP or calcium levels or assays to measure adenylyl cyclase activity. These compounds identified by the above assays may be agonists, antagonists, suppressors, or inducers of the receptor. The functional activity of these compounds is best assessed by using the receptor either natively expressed in tissues or cloned and exogenously  
20 expressed.

Once the receptor is cloned and expressed in a mammalian cell line, such as COS-7 cells or CHO cells, the recombinant receptor is in a well-characterized environment. The membranes from the recombinant cells expressing the receptor are then isolated according to  
25 methods known in the art. The isolated membranes or whole cells may be used in a variety of membrane-based or whole cell-based receptor binding assays. Ligands (either agonists or antagonists) may be identified by standard radioligand binding assays. These assays will measure the intrinsic affinity of the ligand for the receptor. In addition,  
30 the activity of receptor ligands or modulators of the receptor may be measured in functional assays as described above.

The present invention provides methods of identifying compounds that bind to a novel mammalian NPY Yx receptor. Methods of identifying compounds are exemplified by an assay, comprising:

- 23 -

a) cloning DNA which encodes a mammalian neuropeptide Y Y<sub>x</sub> receptor;

b) splicing the DNA into an expression vector to produce a construct such that the NPY Y<sub>x</sub> receptor is operably linked to  
5 transcription and translation signals sufficient to induce expression of the NPY Y<sub>x</sub> receptor upon introduction of the construct into a prokaryotic or eukaryotic cell;

c) introducing the construct into a prokaryotic or eukaryotic cell which does not express the NPY Y<sub>x</sub> receptor in the  
10 absence of the introduced construct; and

d) incubating cells or membranes isolated from cells produced in step c with a quantifiable compound known to bind to the NPY Y<sub>x</sub> receptor, and subsequently adding test compounds at a range of concentrations so that the test compounds compete with the quantifiable  
15 compound for the NPY Y<sub>x</sub> receptor, such that an IC<sub>50</sub> for the test compound is obtained as the concentration of test compound at which 50% of the quantifiable compound becomes displaced from the NPY Y<sub>x</sub> receptor.

The present invention is also directed to methods for  
20 screening for compounds which modulate the expression of DNA or RNA encoding NPY Y<sub>x</sub> receptor or which modulate the function of NPY Y<sub>x</sub> receptor protein. Compounds which modulate these activities may be DNA, RNA, peptides, proteins, or non-proteinaceous organic molecules. Compounds may modulate by  
25 increasing or attenuating the expression of DNA or RNA encoding receptor, or the function of receptor protein. Compounds that modulate the expression of DNA or RNA encoding receptor or the function of receptor protein may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there  
30 is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample.

The present invention is also directed to methods for screening for compounds which modulate the expression of DNA or

- 24 -

RNA encoding the NPY Y<sub>x</sub> receptor as well as the function of the NPY Y<sub>x</sub> receptor protein *in vivo*. Compounds which modulate these activities may be DNA, RNA, peptides, proteins, or non-proteinaceous organic molecules. Compounds may modulate by  
5 increasing or attenuating the expression of DNA or RNA encoding the receptor, or the function of the receptor protein. Compounds that modulate the expression of DNA or RNA encoding the receptor or the function of the receptor protein may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine  
10 whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample.

Kits containing receptor DNA, antibodies to receptor, or receptor protein may be prepared. Such kits are used to detect  
15 DNA which hybridizes to receptor DNA or to detect the presence of receptor protein or peptide fragments in a sample. Such characterization is useful for a variety of purposes including but not limited to forensic, taxonomic or epidemiological studies.

The DNA molecules, RNA molecules, recombinant protein and antibodies of the present invention may be used to screen and measure levels of receptor DNA, receptor RNA or receptor protein. The recombinant proteins, DNA molecules, RNA molecules and antibodies lend themselves to the formulation of kits suitable for  
20 the detection and typing of receptor. Such a kit would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as recombinant receptor protein or anti-receptor antibodies suitable for detecting receptor. The carrier may also contain a  
25 means for detection such as labeled antigen or enzyme substrates or  
30 the like.

Compounds identified by the screening methods identified herein are formulated into pharmaceutical compositions according to standard methods. The compounds or pharmaceutical compositions are

- 25 -

used either alone or in combination with other compounds or compositions for the treatment of animals (including humans) in need of treatment. Conditions which can be treated with compounds identified by the methods of the present invention include but are not limited to  
5 obesity, regulation of appetite, congestive heart failure, diabetes, anxiety, hypertension, cocaine withdrawal, congestive heart failure, memory enhancement, cardiac and cerebral vasospasm, pheochromocytoma and ganglioneuroblastoma, and Huntington's, Alzheimer's and Parkinson's diseases. Thus, animals (including  
10 humans) having a condition, the condition being characterized by factors selected from altered levels of neuropeptide Y, altered activities of neuropeptide Y, altered levels of neuropeptide Y receptor activity, altered neuropeptide Y receptor activity, and combinations thereof, can be treated with compounds or derivatives of compounds (or  
15 pharmaceutical compositions comprising the compounds or derivatives of compounds) identified by the screening methods described herein.

Pharmaceutically useful compositions comprising modulators of receptor activity, may be formulated according to known methods such as by the admixture of a pharmaceutically  
20 acceptable carrier. Examples of such carriers and methods of formulation may be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain a  
25 pharmacologically effective amount of the protein, DNA, RNA, or modulator. The term "pharmacologically effective amount," as used herein, means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response that is being sought by a researcher or clinician.

Therapeutic or diagnostic compositions of the invention  
30 are administered to an individual in amounts sufficient to treat or diagnose disorders. The effective amount may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration.

- 26 -

The pharmaceutical compositions may be provided to the individual by a variety of routes such as subcutaneous, topical, oral and intramuscular.

The terms "derivative" or "chemical derivative" describe a molecule that contains additional chemical moieties which are not normally a part of the base molecule. Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Examples of such moieties are described in a variety of texts, such as Remington's Pharmaceutical Sciences.

Compounds identified according to the methods disclosed herein may be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents may be desirable.

The present invention also has the objective of providing suitable topical, oral, systemic and parenteral pharmaceutical formulations for use in the novel methods of treatment of the present invention. The compositions containing compounds identified according to this invention as the active ingredient can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compounds identified by the methods of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, these compounds can

- 27 -

be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery  
5 system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

For combination treatment with more than one active agent, the individual components of the combination can be administered separately at different times during the course of  
10 therapy or concurrently in divided or single combination forms. The instant invention is therefore to be understood as embracing all such regimes of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly.

The dosage regimen utilizing the compounds of the  
15 present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the  
20 particular compound thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of drug within the range that yields efficacy without  
25 toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

The following examples are provided to further define the invention without, however, limiting the invention to the particulars of the examples.

- 28 -

EXAMPLE 1Genomic DNA Cloning of a NPY Yx Receptor

A mouse genomic DNA library was constructed using published procedures (Mudgett, J.S. and MacInnes, M.A. *Genomics* 8, 623-633(1990)) in the cosmid sCOS vector using genomic DNA isolated from the embryonic stem cell line J1 (ES-J1) (Dr. R. Jaenisch, The Whitehead Institute) which was derived from J129 SV mice (J. Mudgett, MRL). The recombinant DNA library constructed from genomic DNA of ES-J1 cells was plated on Colony/Plaque screen hybridization transfer membrane (Dupont/NEN) at a density of approximately 30,000 colonies per plate. Alternatively, ES-14TG2A mouse pluripotent stem cells (ATCC CRL1821) can be used in place of the ES-J1 stem cell line. Replicas of master plates were lysed and processed for hybridization using standard protocols (Sambrook, J., Fritsch, E.F., Maniatis, T. in *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989). The DNA was UV crosslinked to the membrane with a Stratalinker (Stratagene). The filters were incubated overnight at 42°C with radiolabeled probe in 35% formamide hybridization solution, [5 x SSC, 0.02% SDS, 0.1% n-lauroyl sarcosine, 0.02% (w/v) blocking buffer (Boehringer Mannheim Biochemicals)]. The probe, a DNA fragment containing coding sequences of the human Y1 neuropeptide Y receptor from transmembrane domains 3 to 7, was generated by PCR (Perkin Elmer Cetus) using primers 554 (5' CACTGGGTCTTTGGTGAGGCGATGTG 3') (SEQ. I.D. NO. 1) and 1300 (5' CCCATAAAATATGGGGTTGACACAAGTGG 3') (SEQ. I.D. NO. 2) with human Y1 cDNA as template and in the presence of [ $\alpha$ -<sup>32</sup>P] dCTP (deoxycytidinetriphosphate) (3000 Ci/mmol). Filters were washed at a final stringency of 0.5 x SSC, 0.1% SDS at 42°C. Positives were rescreened to isolate single colonies. DNA was prepared from positive colonies, digested with restriction enzymes, and Southern blot analysis was done to identify restriction fragments for subcloning.

- 29 -

A fragment identified by this hybridization was subcloned into pGEM-3ZF (Promega).

## EXAMPLE 2

5

### Isolation of ET15952 (pVE2841)

Filters containing *Pst*I fragments cloned from the mouse cosmid library were hybridized to a human NPY Y1 receptor cDNA probe. The probe was prepared using a PCR labeling kit (Bethesda Research Labs, Bethesda, MD). The forward primer was (5' TTGGCCATGATATTTACCTTAGCT 3') (SEQ. I.D. NO. 3), the reverse primer was (5' GCATCAAGTGTTACATTTTGGAAAC 3') (SEQ. I.D. NO. 4), and the template was a 928 bp EcoRI-EcoRV restriction fragment prepared with Qiaex resin (Qiagen Inc., Chatsworth, CA) from an agarose gel slice of restricted and electrophoresed NPY Y1 cDNA. The PCR product was a 463 bp fragment spanning transmembrane domains 1 to 4 of the NPY Y1 receptor. Hybridization was performed using prehybridization and hybridization solutions (5 Prime -> 3 Prime, Inc., Boulder, CO) with an Autoblots oven and bottles (Bellco Glass Co., Vineland, NJ). After a prehybridization at 60°C for 3 hrs,  $2 \times 10^7$  CPM of the heat denatured probe was added to each hybridization bottle and the filters were hybridized at 60°C for 1 hr, then 55°C for 11 hrs, and 50°C for 3 hrs. After hybridization, the filters were washed in 2 X SSC, 0.1 % SDS, first at room temperature for 60 min, then at 30°C for 20 min, then at 45°C for 30 min and the filters were then exposed to Kodak X-OMAT AR film for 4 hrs. Several putative hybridizing colonies were identified and DNA was purified from these clones by Qiaprep system (Qiagen, Inc., Chatsworth, CA), subjected to digestion with EcoRI and EcoRV, separated by electrophoresis in an agarose gel, and transferred to a nylon filter (Zeta-Probe, Bio-Rad Laboratories, Hercules, CA) using the Stratagene Posiblots system. The resulting filters were hybridized to the PCR labeled 463 bp NPY Y1 receptor cDNA fragment as described above. After a final stringent wash at 55°C for 45 min in 2 X SSC, 0.1 SDS the filters were then exposed to Kodak X-

- 30 -

OMAT AR film for 1 hr. One hybridizing clone, designated ET15952 and containing plasmid pVE2841, was selected for DNA sequence analysis.

5

### EXAMPLE 3

#### Sequence Analysis of pVE2841

DNA was prepared from overnight cultures of ET15952 using the Wizard DNA Purification System (Promega Corp., Madison, WI) and subjected to automated sequence analysis using the PRISM Dye Deoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, CA). Initial sequencing primers were complementary to the T7 and SP6 promoter sites in pGEM 3ZF, additional primers were made complementary to the insert DNA in pVE2841. Sequencing indicated that pVE2841 contains a 2280 bp PstI insert. FRAMES analysis using the Genetics Computing Group software (Madison, WI) identified a 371 amino acid open reading frame (Fig. 1). Hydrophobicity analysis using PEPLOT (Genetics Computing Group software (Madison, WI)) indicates the open reading frame has 7 hydrophobic domains characteristic of the transmembrane spanning domains of G-protein coupled receptors (Fig. 2). The DNA sequence of the PstI fragment and the region encoding the open reading frame are shown in Fig. 3. The DNA encoding the open reading frame appears to lack introns and is 59% identical to mouse NPY Y1 receptor cDNA, while the codon sequence of the open reading frame is 52% identical to the mouse NPY Y1 receptor. A comparative alignment determined by BESTFIT (Genetics Computing Group software (Madison, WI)) of the mouse NPY Y1 receptor and the novel homolog described herein is presented in Fig. 4. In Fig. 4 the seven potential transmembrane spanning domains are indicated as are three putative N-linked glycosylation sites (indicated by a \*).

- 31 -

EXAMPLE 4Construction of a Vector for Expression of the murine NPY Y<sub>x</sub> Receptor DNA in Mammalian Cells

5           3 µg of pcDNA3 DNA (Invitrogen Corp., San Diego, CA) was digested with restriction enzyme *EcoRV* according to the manufacturer's directions (Bethesda Research Labs, Bethesda, MD) in a reaction mixture of 40 µl. After 2 hours of digestion at 37°C, the 5' ends of the vector were dephosphorylated by adding 2 µl of an  
10 alkaline phosphatase solution (Boehringer Mannheim, Indianapolis, IN) (0.1 U in 10 mM Tris pH7, 10 mM MgCl<sub>2</sub>, 0.1 mM ZnCl<sub>2</sub>, 50% glycerol) and incubating at 37°C. After an hour, the enzymes were heat inactivated at 65°C for 10 min. 5 µg of pVE2841 were digested with restriction enzyme *PstI*, according to the manufacture's  
15 directions (Bethesda Research Labs, Bethesda, MD), in a 40 µL solution. The 3' overhanging ends of the 2.3 kb *PstI* fragment were converted to blunt ends by adding 8.8 µL of 5x T4 DNA Polymerase buffer and 5 U of T4 DNA polymerase (Bethesda Research Labs, Bethesda, MD) and continuing the incubation at 37°C. After an  
20 hour, the enzymes were heat inactivated at 65°C for 10 min. The reaction products were separated on a 1% agarose gel (Sigma Chemical Co., St. Louis MO) and the cleaved pcDNA3 vector and 2.3 kb fragment were purified from the agarose gel using Qiaex resin (Qiagen Corp., Chatsworth, CA). The purified DNA  
25 fragments were resuspended in 25 µL of TE (10 mM Tris pH 7.4, 1 mM EDTA). The 2.3 kb fragment was ligated to the pcDNA3 vector DNA in a 20 µL reaction containing 1 µL of purified vector, 3 µL of purified 2.3 kb fragment, 4 µL of 5x T4 DNA ligation buffer (Bethesda Research Labs, Bethesda, MD), 11 µL of water, and 1 µl of T4 DNA ligase (1U, Bethesda Research Labs, Bethesda,  
30 MD). After ligation for 2 hr at 20°C, 1 µL of the ligation mixture was transformed into 20 µL of XL1Blue competent *E. coli* cells (Stratagene, La Jolla, CA), according to the manufacturer's directions. Transformants were isolated as ampicillin-resistant

- 32 -

colonies and plasmid DNA was isolated from transformants by Qiaprep system (Qiagen Corp. Chatsworth, CA). Among the transformants containing the 2.3 kb insert, one isolate was identified and designated pVE2863 which contained the 2.3 kb in the correct orientation for expression from the CMV promoter.

### EXAMPLE 5

#### Construction of a second Vector for Expression of the murine NPY Yx Receptor DNA in Mammalian Cells

3 µg of pCI-neo (Promega corp., Madison, WI) was digested with restriction enzymes EcoRI and XbaI according to the manufacturer's directions (Bethesda Research Labs, Bethesda, MD) in a reaction mixture of 50 µL. After 16 hours of digestion at 37°C, the 5' ends of the vector were dephosphorylated by adding 2 µL of an alkaline phosphatase solution (Boehringer Mannheim, Indianapolis, IN) (0.1 U in 10 mM Tris pH7, 10 mM MgCl<sub>2</sub>, 0.1 mM ZnCl<sub>2</sub>, 50% glycerol) and incubating at 37°C. After an hour of incubation, the DNA was purified using a Wizard PCR Preps kit (Promega corp., Madison, WI) and resuspended in 50 µL water. A fragment of the Yx gene containing an EcoRI restriction site 5' to the ATG, and an XbaI restriction site 3' to the stop codon was generated by polymerase chain reaction (PCR). Ten ng of PVE2841 was used in a 50 µL reaction containing 5 units of Pfu polymerase (Promega corp., Madison, WI) and 20 pmoles each of primers MNPY5FRI (5'GATCGAATTCG CCATGGAAGTGCTCACAAAC 3') (SEQ. I.D. NO. 13) and MNPY5RXBA (5'GATCTCTAGACTA TATGCCTGTTGGTATGTG 3') (SEQ. I.D. NO. 14) in buffer supplied by the manufacturer. The reactions were carried out for 25 cycles of 95 degrees for 1 minute, 50 degrees for 30 seconds, and 72 degrees for 1 minute 30 seconds. The products of the reaction were resolved on a 1% agarose gel and the single predominant product was isolated and purified using a Wizard PCR Preps kit (Promega Corp., Madison, WI). The purified PCR fragment was digested in a

- 33 -

50  $\mu$ L reaction containing 10 units of EcoRI and 10 units of XbaI according to the manufacturer's directions (Bethesda Research Labs, Bethesda, MD) for 16 hours. The PCR fragment was repurified as before using the Wizard PCR Preps kit and resuspended in 50  $\mu$ L of water. The PCR fragment was ligated to the pCI-neo vector DNA in a 10  $\mu$ L reaction containing 1  $\mu$ L of purified vector, 3  $\mu$ L of purified PCR fragment, 2  $\mu$ L of 5x T4 DNA ligation buffer (Bethesda Research Labs, Bethesda, MD), 3  $\mu$ L of water, and 1  $\mu$ L of T4 DNA ligase (1U, Bethesda Research Labs, Bethesda, MD). After ligation for 16 hr at 12°C, 1  $\mu$ L of the ligation mixture was transformed into 50  $\mu$ L of Max-Efficiency DH5 alpha competent *E. coli* cells (Bethesda Research Labs, Bethesda, MD), according to the manufacturer's directions. Transformants were isolated as ampicillin-resistant colonies and plasmid DNA was isolated from transformants by Qiaprep system (Qiagen Corp. Chatsworth, CA). Transformants containing the appropriate fragment insert were identified by restriction with EcoRI and Xba I. Fidelity of the Yx gene was verified by automated sequence analysis using the PRISM Dye Deoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, CA). A single clone was selected and designated pCI-mY5.

#### EXAMPLE 6

##### 25 Pharmacology of the Recombinant NPY Yx Receptor

Using electroporation, COS-7 cells were transfected with the pcDNA3 or the pCI-neo expression vector containing the genomic DNA for the murine NPY Yx receptor (pVE2863 or pCI-mY5, respectively). Two days post transfection, cells were dissociated with enzyme-free dissociation buffer, centrifuged at 1000 rpm for 10 minutes at 4°C, and resuspended in 10 mM Tris, pH 7.4 containing 0.1 M PMSF (phenylmethylsulfonyl fluoride), 10  $\mu$ M phosphoramidon, and 40  $\mu$ g/ml bacitracin. The cell suspension was homogenized with a glass homogenizer, and centrifuged at 2200 rpm

- 34 -

for 10 minutes at 4°C to remove cell debris. The plasma membrane fraction was recovered by centrifuging the supernatant at 18,000 rpm in a Sorvall SS-34 rotor for 15 minutes at 4°C. The membrane pellet was resuspended in the Tris/PMSF/phosphoramidon/bacitracin buffer by trituration 5 times using a 25g needle and syringe. The membranes were stored frozen at -80°C until use.

Membranes (50 µg protein) were incubated with <sup>125</sup>I-PYY (90 pM) in the presence or absence of 1µM unlabeled PYY (non-specific binding) or serial dilutions of other competing peptides in 0.25 ml of 50 mM Tris, pH 7.4 containing 2 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 5 mM KCl, 0.2% bovine serum albumin, 10 µM phosphoramidon, 4 µg/ml leupeptin and 40 µg/ml bacitracin. After 2 hours at room temperature, the incubation was filtered over GF/C filters, and the radioactivity bound to the filters was quantified with a Packard gamma counter. The percentage of inhibition of <sup>125</sup>I-PYY binding at a given concentration of competing peptide was plotted versus the concentration of peptide added, and the concentration of peptide causing 50% inhibition of binding (IC<sub>50</sub>) was determined using non-linear regression performed by PRISM (GraphPad).

<u>Competitor</u>	<u>IC<sub>50</sub> (nM)</u>
PYY	14
NPY	6
[Leu <sup>31</sup> , Pro <sup>34</sup> ]NPY	9
NPY(2-36)	16
NPY(13-36)	134

### EXAMPLE 7

#### 25 Human NPY Y<sub>x</sub> Receptor

Isolation of the hybridization probe. A hybridization probe with identity to the human NPY Y<sub>x</sub> receptor was generated by PCR using human genomic DNA as the target. Oligonucleotide primer pairs based upon the DNA

- 35 -

sequence of the mouse NPY Yx receptor (primer 1; 5'ACCAGTGGCAAGAGCAACAAC (SEQ. I.D. NO. 8), primer 2; 5'CTCATTGGTGAGGTGGTAGGAC (SEQ. I.D. NO. 9)) were used in a primary PCR reaction. The products of this reaction were used as the  
5 template for a second PCR reaction using primer 2 and primer 3 (5'.GGGCATTTTTGGAAACCTCTC (SEQ. I.D. NO. 10)). Primer 3, a 'nested' primer, was designed to hybridize to mouse NPY Yx receptor sequences which are internal to those with homology to primers 1 and 2. A product obtained from the second PCR reaction was cloned and found to  
10 share homology to the mouse NPY Yx receptor by DNA sequence analysis in both directions using an ABI 373A automated sequencing unit (Perkin Elmer).

Isolation of a human NPY Yx receptor cDNA clone. A commercial  
15 lambda phage library (Lambda Zap2, Stratagene) containing cDNAs derived from human heart mRNA was screened. Approximately  $1 \times 10^6$  plaques were plated at a density of 50,000 plaques per 150 mm plate and transferred to Colony/Plaque screen hybridization membranes (Dupont/NEN) which were then processed by standard protocols (as  
20 supplied by Stratagene). DNA was UV crosslinked to the membrane with a Stratalinker (Stratagene). The filters were prewashed with 500 mls of 0.1X SSC, 0.1% SDS at 65°C for 1 hour and then prehybridized in a solution of 0.25M NaPO<sub>4</sub>, 7% SDS pH7.5 at 60°C for 1 hour. The  
25 PCR fragment having identity to the human NPY Yx receptor described above was labeled by random priming (Rediprime, Amersham) in the presence of [ $\alpha$ -<sup>32</sup>P]dCTP (3000 Ci/mmole). The membranes were allowed to hybridize to the [ $\alpha$ -<sup>32</sup>P]-labeled PCR fragment in a solution of 0.25M NaPO<sub>4</sub>, 7% SDS, 10% dextran sulfate pH7.5 for 16 hours at  
30 60°C. The membranes were washed in 1 liter of 1X SSC, 0.1% SDS at 45°C and then exposed to Kodak XAR-5 Xray film. Primary positives identified by this procedure were selected, re-plated, and subjected to a second round of hybridization screening to allow identification and selection of individual hybridization positive plaques. Plasmids containing the cDNA inserts were rescued from the phage using

- 36 -

methodology supplied by the manufacturer (Stratagene) which results in the generation of bacterial colonies. Bacterial colonies with plasmids containing the human NPY Yx receptor cDNA sequences were identified by filter hybridization with the [ $\alpha$ -<sup>32</sup>P]-labeled human NPY Yx receptor probe using standard methodologies (Sambrook, J., Fritsch, E.F., Maniatis, T. in *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989). The cloned cDNA, pCD13, was analyzed by sequencing in both directions using an ABI 373A automated sequencing unit (Perkin Elmer).

Structure of the human NPY Yx receptor cDNA.

The DNA sequence of the approximately 1499 bp clone of human NPY Yx receptor cDNA, pCD13 (SEQ. I.D. NO. 11) contains an open reading frame of about 1110 bp. The proposed initiator methionine at nucleotide 182 conforms to the Kozak sequence (M. Kozak, *Journal of Cell Biology* **108**, 229-241 (1989)) at the +4 position but not at the -3 position. The proposed 370 amino acid sequence (SEQ. I.D. NO. 12) encoded by the open reading frame is 82% identical to the amino acid sequence encoded by the open reading frame of the mouse NPY Yx receptor clone. The human NPY Yx receptor cDNA is 78% identical to the DNA of the mouse NPY Yx receptor. The human NPY Yx receptor cDNA described herein contains 181 untranslated nucleotides at the 5 prime end and 208 nucleotides following the stop codon.

- 37 -

EXAMPLE 8Construction of a Vector for Expression of the Human NPY Yx Receptor DNA in Mammalian Cells

5                   2 µg of pcDNA3 DNA (Invitrogen Corp., San Diego, CA) was digested with restriction enzyme *EcoRI* according to the manufacturer's directions (Bethesda Research Labs, Bethesda, MD) in a reaction mixture of 20 µl. The DNA was resolved by electrophoresis on a 1% agarose gel (Sigma Chemical Co., St. Louis MO) and the linearized vector was identified and excised from the gel. The DNA was purified by Gene Clean using the procedure supplied by the manufacturer (BIO 101 Inc., Vista, CA). The 5' ends of the vector were dephosphorylated by adding 1 µl (0.1 unit) of an alkaline phosphatase solution (Boehringer Mannheim, Indianapolis, IN) in 50 µl of 10 mM Tris pH7, 10 mM MgCl<sub>2</sub>, 0.1 mM ZnCl<sub>2</sub>, 50% glycerol and incubating at 37°C for 1 hour. The DNA was subjected to a second round of Gene Clean purification and resuspended in 50 µl of TE (10 mM Tris pH 7.4, 1 mM EDTA). Approximately 2 µg of an isolated human NPYx receptor clone, pCD13, was digested with restriction enzyme *EcoRI* (Bethesda Research Labs, Bethesda, MD) in a 20 µl solution. The reaction products were resolved by electrophoresis on a 1% agarose gel and the approximately 1.6kb fragment containing the receptor coding sequences was purified by Gene Clean as above and resuspended in 50 µl of TE. The 1.6 kb fragment was ligated to the linearized pcDNA3 vector DNA in a 10 µl reaction containing 1 µl of purified vector, 3 µl of purified 1.6 kb fragment, 1 µl of 10x T4 DNA ligation buffer (Boehringer Mannheim, Indianapolis, IN), 5 µl of water, and 1 µl of T4 DNA ligase (1U, Boehringer Mannheim, Indianapolis, IN). After ligation for 2 hr at 20°C, 1 µl of the ligation mixture was transformed into 50 µl of Max Efficiency DH5alpha competent *E. coli* cells (Bethesda Research Labs, Bethesda, MD), according to the manufacturer's directions. Transformants were isolated as ampicillin-resistant colonies and plasmid DNA was

- 38 -

isolated from transformants using the WIZARD miniprep system (Promega Corporation, Madison WI). Among the transformants containing the 1.6kb insert, one isolate was identified and designated pcDNA3HYx-RI, which contained the 1.6 kb in the correct orientation for expression from the CMV promoter.

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In an analagous fashion, 2 µg of pcDNA3 DNA (Invitrogen Corp., San Diego, CA) was digested with restriction enzymes *EcoRI* and *Hind III* according to the manufacturer's directions (Bethesda Research Labs, Bethesda, MD) in a reaction mixture of 20 µl. The large fragment of doubly digested vector was purified by Gene Clean and resuspended in 50 µl of TE. Approximately 2 µg of one of the isolated human NPYx receptor clones, pCD13, was digested with restriction enzymes *EcoRI* and *Hind III* according to the manufacturer's directions (Bethesda Research Labs, Bethesda, MD) in a 20 µl reaction. The approximately 1.6kb fragment containing the receptor coding sequences was purified by Gene Clean and resuspended in 50 µl of TE. The 1.6 kb fragment was ligated to the pcDNA3 vector DNA in a 10 µl reaction containing 1 µl of purified vector, 3 µl of purified 1.6 kb fragment, 1 µl of 10x T4 DNA ligation buffer (Boehringer Mannheim, Indianapolis, IN), 5 µl of water, and 1 µl of T4 DNA ligase (1U, Boehringer Mannheim, Indianapolis, IN). After ligation for 2 hr at 20°C, 1 µl of the ligation mixture was transformed into 50 µl of Max Efficiency DH5alpha competent *E. coli* cells (Bethesda Research Labs, Bethesda, MD), according to the manufacturer's directions. Transformants were isolated as ampicillin-resistant colonies and plasmid DNA was isolated from transformants using the WIZARD miniprep system (Promega Corporation, Madison WI). Among the transformants containing the 1.6 kb insert, one isolate was identified and designated pcDNA3HYx-Hind/RI, which contained the 1.6 kb in the correct orientation for expression from the CMV promoter. The two expression constructs, pcDNA3HYx-RI and pcDNA3HYx-Hind/RI, differ in the amount of cDNA sequences 5 prime to the

- 39 -

ATG translational start codon which remain in the final expression construct.

#### EXAMPLE 9

##### 5 Cloning and Expression of NPY Yx Receptor DNA into Bacterial Expression Vectors

Recombinant receptor is produced in a bacterial expression system such as E. coli. The receptor expression cassette is transferred into an E. coli expression vector; expression vectors include but are not limited to, the pET series (Novagen). The pET  
10 vectors place receptor expression under control of the tightly regulated bacteriophage T7 promoter. Following transfer of this construct into an E. coli host which contains a chromosomal copy of the T7 RNA polymerase gene driven by the inducible lac promoter, expression of receptor is induced by addition of an appropriate lac  
15 substrate (IPTG) added to the culture. The levels of expressed receptor are determined by the assays described herein.

#### EXAMPLE 10

##### 20 Cloning and Expression of NPY Yx Receptor DNA into a Vector for Expression in Insect Cells

Baculovirus vectors derived from the genome of the AcNPV virus are designed to provide high level expression of DNA in the Sf9 line of insect cells (ATCC CRL# 1711). Recombinant  
25 baculovirus expressing receptor DNA is produced by the following standard methods (InVitrogen Maxbac Manual): the receptor DNA constructs are ligated into the polyhedrin gene in a variety of baculovirus transfer vectors, including the pAC360 and the BlueBac vector (InVitrogen). Recombinant baculoviruses are generated by  
30 homologous recombination following co-transfection of the baculovirus transfer vector and linearized AcNPV genomic DNA [Kitts, P.A., *Nuc. Acid. Res.* 18, 5667 (1990)] into Sf9 cells. Recombinant pAC360 viruses are identified by the absence of inclusion bodies in infected cells and recombinant pBlueBac viruses

- 40 -

are identified on the basis of  $\beta$ -galactosidase expression (Summers, M. D. and Smith, G. E., Texas Agriculture Exp. Station Bulletin No. 1555). Following plaque purification, receptor expression is measured by assays described herein.

5                    Authentic receptor is found in association with the infected cells. Active receptor is extracted from infected cells by hypotonic or detergent lysis.

                    Alternatively, the receptor is expressed in the *Drosophila* Schneider 2 cell line by cotransfection of the Schneider 2  
10 cells with a vector containing the receptor DNA downstream and under control of an inducible metallothionin promoter, and a vector encoding the G418 resistant neomycin gene. Following growth in the presence of G418, resistant cells are obtained and induced to express receptor by the addition of CuSO<sub>4</sub>. Identification of  
15 modulators of the receptor is accomplished by assays using either whole cells or membrane preparations.

#### EXAMPLE 11

##### Cloning of NPY Y<sub>x</sub> Receptor DNA into a Yeast Expression Vector

20                    Recombinant receptor is produced in the yeast *S. cerevisiae* following the insertion of the receptor DNA cistron into expression vectors designed to direct the intracellular or extracellular expression of heterologous proteins. In the case of intracellular expression, vectors such as EmBLyex4 or the like are  
25 ligated to the receptor cistron [Rinas, U. et al., *Biotechnology* 8, 543-545 (1990); Horowitz B. et al., *J. Biol. Chem.* 265, 4189-4192 (1989)]. For extracellular expression, the receptor cistron is ligated into yeast expression vectors which fuse a secretion signal. The levels of expressed receptor are determined by the assays described  
30 herein.

- 41 -

### EXAMPLE 12

#### Purification of Recombinant NPY Yx Receptor

Recombinantly produced receptor may be purified by a variety of procedures, including but not limited to antibody affinity  
5 chromatography.

Receptor antibody affinity columns are made by adding the anti-receptor antibodies to Affigel-10 (Biorad), a gel support which is pre-activated with N-hydroxysuccinimide esters such that the antibodies form covalent linkages with the agarose gel bead  
10 support. The antibodies are then coupled to the gel via amide bonds with the spacer arm. The remaining activated esters are then quenched with 1 M ethanolamine HCl (pH 8). The column is washed with water followed by 0.23 M glycine HCl (pH 2.6) to remove any non-conjugated antibody or extraneous protein. The column is then  
15 equilibrated in phosphate buffered saline (pH 7.3) together with appropriate membrane solubilizing agents such as detergents, and the cell culture supernatants or cell extracts containing solubilized receptor or receptor subunits are slowly passed through the column. The column is then washed with phosphate-buffered saline (PBS)  
20 supplemented with detergents until the optical density (A<sub>280</sub>) falls to background; then the protein is eluted with 0.23 M glycine-HCl (pH 2.6) supplemented with detergents. The purified receptor protein is then dialyzed against PBS.

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### EXAMPLE 13

#### Screening Assays

Recombinant cells containing DNA encoding the novel murine or human NPY Yx receptor, membranes derived from the recombinant cells, or recombinant receptor preparations derived from  
30 the cells or membranes may be used to identify compounds that modulate the murine or human NPY Yx receptor activity. Modulation of such activity may occur at the level of DNA, RNA, protein or combinations thereof. One method of identifying compounds that

- 42 -

modulate NPY Y<sub>x</sub> receptor activity or modulate binding of ligands to the protein, comprises:

- 5 (a) mixing a test compound with a solution containing NPY Y<sub>x</sub> receptor, or a functional derivative thereof, to form a mixture;
- (b) measuring NPY Y<sub>x</sub> receptor activity in the mixture; and
- 10 (c) comparing the NPY Y<sub>x</sub> receptor activity of the mixture to a standard. Preferably, the neuropeptide Y Y<sub>x</sub> receptor, or functional derivative thereof, is characterized by a pharmacological binding profile with affinities of PYY ≅ NPY ≅ [Leu<sup>31</sup>Pro<sup>34</sup>]NPY ≅ NPY(2-36) > NPY(13-36).

15 This screening assay will detect a compound that has affinity for the NPY Y<sub>x</sub> receptor. Such compounds may be either agonists or antagonists and may be peptides, proteins, or non-proteinaceous organic molecules.

Another method of identifying compounds that modulate the NPY Y<sub>x</sub> receptor, comprises:

- 20 (a) mixing a test compound with cells which express the NPY Y<sub>x</sub> receptor to form a mixture;
- (b) culturing the cells in the presence of the test compound;
- (c) measuring NPY Y<sub>x</sub> receptor or second messenger activity in the mixture; and
- 25 (d) comparing the NPY Y<sub>x</sub> receptor activity of the mixture to a standard. In a preferred embodiment, the cells are transformed by an expression vector containing a sequence selected from a sequence as shown in Figure 3, a sequence of substantial homology to the sequence shown in Figure 3, a fragment of the sequence shown in Figure 3, a fragment of the sequence of substantial homology to the sequence shown in Figure 3, a sequence as shown in
- 30 Figure 5, a sequence of substantial homology to the sequence shown in Figure 5, a fragment of the sequence shown in Figure 5 or a fragment

- 43 -

of the sequence of substantial homology to the sequence shown in Figure 5.

In one example of the method, the expression vector contains a DNA sequence selected from a DNA sequence having a sequence selected from a sequence as shown in Figure 3, a sequence of substantial homology to the sequence shown in Figure 3, a fragment of the sequence shown in Figure 3 or a fragment of the sequence of substantial homology to the sequence shown in Figure 3. Preferably, the expression vector contains a DNA sequence selected from the sequence shown in Figure 3, the sequence of substantial homology to the sequence shown in Figure 3 or the fragment of the sequence shown in Figure 3 comprising bases 822 to 1934.

In another example of the method, the expression vector contains a DNA sequence selected from a DNA sequence having a sequence selected from a sequence as shown in Figure 5, a sequence of substantial homology to the sequence shown in Figure 5, a fragment of the sequence shown in Figure 5 or a fragment of the sequence of substantial homology to the sequence shown in Figure 5. Preferably, the expression vector contains a DNA sequence selected from the sequence shown in Figure 5, the sequence of substantial homology to the sequence shown in Figure 5 or the fragment of the sequence shown in Figure 5 comprising bases 182 to 1291.

This screening assay will detect a compound which modulates NPY Y<sub>x</sub> receptor activity. Such compounds may be either agonists, antagonists, suppressors or inducers. Such compounds may be DNA, RNA, peptides, proteins, or non-proteinaceous organic molecules.

While the invention has been described and illustrated with reference to certain preferred embodiments thereof, those skilled in the art will appreciate that various changes, modifications and substitutions can be made therein without departing from the spirit and scope of the invention. It is intended, therefore, that the invention be limited only by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.

- 44 -

## SEQUENCE LISTING

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  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 08/383,746
  - (B) FILING DATE: 03-FEB-1995
- (vii) PRIOR APPLICATION DATA:
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## (2) INFORMATION FOR SEQ ID NO:1:

- 45 -

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 26 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CACTGGGTCT TTGGTGAGGC GATGTG

26

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 29 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCCATAAAAT ATGGGGTTGA CACAAGTGG

29

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTGGCCATGA TATTTACCTT AGCT

24

(2) INFORMATION FOR SEQ ID NO:4:

- 46 -

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCATCAAGTG TTACATTTTG GAAC

24

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2280 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 822..1937

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTGCAGTCTA TTGGATGAAG AGTGTACATA TTCATATAAT TCTTAAAGTA GGCAGAAATT	60
AAAGGGGATG GAAATATATA CTTGTACTGC CTTAGATAGT CACCAGGATG TTGTTACAGT	120
CTTCGTTTAC TGCTTCTGAA GCCTATACTG ATAGAATTAA TAAAATACTG AGAGAGAGAG	180
AGAGGGACAG AGAGAGAGAG GGGGAGAGAG AGAGAGAGAG AGAGAGAGAG AGAGAGAGAG	240
AGAGAGAGAG AGAAGAGAAG AAAACAAGGT AAGCCATCTG CTTAACTTAT GTCCACATTC	300
TCTCAAGAGC ATTGTCCTAT TTGTAGAATT ATCTATATTG TTAAGAATCA TCTCCATTGT	360
TAAGATTTTG TGGGCTGGAG ATCCAGCTCT GTTGATAAAG TGCTTGCCTA ACATGCATGA	420
AGTCCTAGGT TCTATTCCCA AGGCTACATA AAACCTTGTG TTGTGATGAA TGCCTGTAAT	480

CCCAGTACGC AGCAAGGAGA GACAAGGAGG ATCAGAAGCT TAAGGACATC ATTTTGTACA	540
TAGTGAGTTT GAGGAAAGCT GAGGTTACAT GGAACTCTCT CTCTCTCAAA AACAAAACAA	600
AACAAAACAA AACCTTCTAC TAATATTCTG GATTCTGTTT GATTTTTAGG ATCTCAAGAG	660
CATGCTGACG TCATTTATGT GTTTCCATCA GATACAGACA GAGATCATAA ACATTTAACT	720
CATTGATTAT ATGTTGAGAG TTGTCCCTCA AGAACCAATG GCCAACATC CACTGAGGAT	780
ACACGGAAGC TTAGAAAATC TCTAATTAAA ATCCTGACAT A ATG GAA GTG CTC	833
	Met Glu Val Leu
	1
ACA AAC CAG CCA ACA CCT AAT AAA ACC AGT GGC AAG AGC AAC AAC TCG	881
Thr Asn Gln Pro Thr Pro Asn Lys Thr Ser Gly Lys Ser Asn Asn Ser	
5 10 15 20	
GCA TTT TTC TAC TTT GAA TCC TGC CAA CCC CCT TTT CTA GCC ATA CTC	929
Ala Phe Phe Tyr Phe Glu Ser Cys Gln Pro Pro Phe Leu Ala Ile Leu	
25 30 35	
TTG CTA CTC ATA GCA TAT ACT GTG ATC CTA ATC ATG GGC ATT TTT GGA	977
Leu Leu Leu Ile Ala Tyr Thr Val Ile Leu Ile Met Gly Ile Phe Gly	
40 45 50	
AAC CTC TCT CTT ATC ATC ATC ATC TTT AAG AAA CAG AGA GAA GCT CAA	1025
Asn Leu Ser Leu Ile Ile Ile Ile Phe Lys Lys Gln Arg Glu Ala Gln	
55 60 65	
AAT GTT ACC AAC ATA CTG ATT GCC AAC CTG TCC CTC TCT GAC ATC TTG	1073
Asn Val Thr Asn Ile Leu Ile Ala Asn Leu Ser Leu Ser Asp Ile Leu	
70 75 80	
GTG TGT GTC ATG TGC ATC CCT TTT ACG GTC ATC TAC ACT CTG ATG GAC	1121
Val Cys Val Met Cys Ile Pro Phe Thr Val Ile Tyr Thr Leu Met Asp	
85 90 95 100	
CAC TGG GTA TTT GGG AAC ACT ATG TGT AAA CTC ACT TCC TAC GTG CAA	1169
His Trp Val Phe Gly Asn Thr Met Cys Lys Leu Thr Ser Tyr Val Gln	
105 110 115	
AGT GTC TCA GTT TCT GTG TCC ATA TTC TCC CTT GTG TTG ATT GCT ATT	1217
Ser Val Ser Val Ser Val Ser Ile Phe Ser Leu Val Leu Ile Ala Ile	
120 125 130	
GAA CGA TAT CAG CTG ATT GTG AAC CCC CGT GGC TGG AAA CCC AGA GTA	1265
Glu Arg Tyr Gln Leu Ile Val Asn Pro Arg Gly Trp Lys Pro Arg Val	
135 140 145	
GCT CAT GCC TAT TGG GGG ATC ATC TTG ATT TGG CTC ATT TCT CTG ACA	1313
Ala His Ala Tyr Trp Gly Ile Ile Leu Ile Trp Leu Ile Ser Leu Thr	
150 155 160	
TTG TCT ATT CCC TTA TTC CTG TCC TAC CAC CTC ACC AAT GAG CCC TTT	1361
Leu Ser Ile Pro Leu Phe Leu Ser Tyr His Leu Thr Asn Glu Pro Phe	



- 49 -

CTTTCTTTTT TTCCTTTGGA AGAATAATAA CTTAAACAAC CTAGACATCA TTAGTGAGGA 2177  
 AGAGAACAAA AATGAGAGAG CATAACAAGGA CAGCAGAGAT GTCTGGGGTA CAAAATTCAC 2237  
 GTTATTCGCT GGAATAGCTA GAAAGTTATT AGTTGTGCTG CAG 2280

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 372 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Glu Val Leu Thr Asn Gln Pro Thr Pro Asn Lys Thr Ser Gly Lys  
 1 5 10 15  
 Ser Asn Asn Ser Ala Phe Phe Tyr Phe Glu Ser Cys Gln Pro Pro Phe  
 20 25 30  
 Leu Ala Ile Leu Leu Leu Leu Ile Ala Tyr Thr Val Ile Leu Ile Met  
 35 40 45  
 Gly Ile Phe Gly Asn Leu Ser Leu Ile Ile Ile Ile Phe Lys Lys Gln  
 50 55 60  
 Arg Glu Ala Gln Asn Val Thr Asn Ile Leu Ile Ala Asn Leu Ser Leu  
 65 70 75 80  
 Ser Asp Ile Leu Val Cys Val Met Cys Ile Pro Phe Thr Val Ile Tyr  
 85 90 95  
 Thr Leu Met Asp His Trp Val Phe Gly Asn Thr Met Cys Lys Leu Thr  
 100 105 110  
 Ser Tyr Val Gln Ser Val Ser Val Ser Val Ser Ile Phe Ser Leu Val  
 115 120 125  
 Leu Ile Ala Ile Glu Arg Tyr Gln Leu Ile Val Asn Pro Arg Gly Trp  
 130 135 140  
 Lys Pro Arg Val Ala His Ala Tyr Trp Gly Ile Ile Leu Ile Trp Leu  
 145 150 155 160  
 Ile Ser Leu Thr Leu Ser Ile Pro Leu Phe Leu Ser Tyr His Leu Thr  
 165 170 175  
 Asn Glu Pro Phe His Asn Leu Ser Leu Pro Thr Asp Ile Tyr Thr His  
 180 185 190  
 Gln Val Ala Cys Val Glu Ile Trp Pro Ser Lys Leu Asn Gln Leu Leu  
 195 200 205

- 50 -

Phe Ser Thr Ser Leu Phe Met Leu Gln Tyr Phe Val Pro Leu Gly Phe  
 210 215 220

Ile Leu Ile Cys Tyr Leu Lys Ile Val Leu Cys Leu Arg Lys Arg Thr  
 225 230 235 240

Arg Gln Val Asp Arg Arg Lys Glu Asn Lys Ser Arg Leu Asn Glu Asn  
 245 250 255

Lys Arg Val Asn Val Met Leu Ile Ser Ile Val Val Thr Phe Gly Ala  
 260 265 270

Cys Trp Leu Pro Leu Asn Ile Phe Asn Val Ile Phe Asp Trp Tyr His  
 275 280 285

Glu Met Leu Met Ser Cys His His Asp Leu Val Phe Val Val Cys His  
 290 295 300

Leu Ile Ala Met Val Ser Thr Cys Ile Asn Pro Leu Phe Tyr Gly Phe  
 305 310 315 320

Leu Asn Lys Asn Phe Gln Lys Asp Leu Met Met Leu Ile His His Cys  
 325 330 335

Trp Cys Gly Glu Pro Gln Glu Ser Tyr Glu Asn Ile Ala Met Ser Thr  
 340 345 350

Met His Thr Asp Glu Ser Lys Gly Ser Leu Lys Leu Ala His Ile Pro  
 355 360 365

Thr Gly Ile \*  
 370

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 382 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Asn Ser Thr Leu Phe Ser Lys Val Glu Asn His Ser Ile His Tyr  
 1 5 10 15

Asn Ala Ser Glu Asn Ser Pro Leu Leu Ala Phe Glu Asn Asp Asp Cys

- 51 -

	20		25		30										
His	Leu	Pro	Leu	Ala	Val	Ile	Phe	Thr	Leu	Ala	Leu	Ala	Tyr	Gly	Ala
	35						40						45		
Val	Ile	Ile	Leu	Gly	Val	Ser	Gly	Asn	Leu	Ala	Leu	Ile	Ile	Ile	Ile
	50						55					60			
Leu	Lys	Gln	Lys	Glu	Met	Arg	Asn	Val	Thr	Asn	Ile	Leu	Ile	Val	Asn
	65				70					75					80
Leu	Ser	Phe	Ser	Asp	Leu	Leu	Val	Ala	Val	Met	Cys	Leu	Pro	Phe	Thr
				85					90					95	
Phe	Val	Tyr	Thr	Leu	Met	Asp	His	Trp	Val	Phe	Gly	Glu	Thr	Met	Cys
			100					105						110	
Lys	Leu	Asn	Pro	Phe	Val	Gln	Cys	Val	Ser	Ile	Thr	Val	Ser	Ile	Phe
		115					120						125		
Ser	Leu	Val	Leu	Ile	Ala	Val	Glu	Arg	His	Gln	Leu	Ile	Ile	Asn	Pro
	130					135							140		
Arg	Gly	Trp	Arg	Pro	Asn	Asn	Arg	His	Ala	Tyr	Ile	Gly	Ile	Thr	Val
	145				150					155					160
Ile	Trp	Val	Leu	Ala	Val	Ala	Ser	Ser	Leu	Pro	Phe	Val	Ile	Tyr	Gln
				165					170					175	
Ile	Leu	Thr	Asp	Glu	Pro	Phe	Gln	Asn	Val	Ser	Leu	Ala	Ala	Phe	Lys
			180					185						190	
Asp	Lys	Tyr	Val	Cys	Phe	Asp	Lys	Phe	Pro	Ser	Asp	Ser	His	Arg	Leu
		195					200					205			
Ser	Tyr	Thr	Thr	Leu	Leu	Leu	Val	Leu	Gln	Tyr	Phe	Gly	Pro	Leu	Cys
	210					215						220			
Phe	Ile	Phe	Ile	Cys	Tyr	Phe	Lys	Ile	Tyr	Ile	Arg	Leu	Lys	Arg	Arg
	225				230					235					240
Asn	Asn	Met	Met	Asp	Lys	Ile	Arg	Asp	Ser	Lys	Tyr	Arg	Ser	Ser	Glu
				245					250					255	
Thr	Lys	Arg	Ile	Asn	Ile	Met	Leu	Leu	Ser	Ile	Val	Val	Ala	Phe	Ala
			260					265						270	
Val	Cys	Trp	Leu	Pro	Leu	Thr	Ile	Phe	Asn	Thr	Val	Phe	Asp	Trp	Asn
		275					280						285		
His	Gln	Ile	Ile	Ala	Thr	Cys	Asn	His	Asn	Leu	Leu	Phe	Leu	Leu	Cys
	290					295					300				
His	Leu	Thr	Ala	Met	Ile	Ser	Thr	Cys	Val	Asn	Pro	Ile	Phe	Tyr	Gly
	305				310					315					320

- 52 -

Phe Leu Asn Lys Asn Phe Gln Arg Asp Leu Gln Phe Phe Phe Asn Phe  
 325 330 335  
 Cys Asp Phe Arg Ser Arg Asp Asp Asp Tyr Glu Thr Ile Ala Met Ser  
 340 345  
 Thr Met His Thr Asp Val Ser Lys Thr Ser Leu Lys Gln Ala Ser Pro  
 355 360 365  
 Val Ala Phe Lys Lys Ile Ser Met Asn Asp Asn Glu Lys Val  
 370 375 380

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ACCAGTGGCA AGAGCAACAA C

21

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTCATTGGTG AGGTGGTAGG AC

22

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGGCATTTTT GGAAACCTCT C

21

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1499 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCCGGGCTG CAGGAATTCC CACATGTTTC CATCAAATAC AGACACAGAT CAGGGAAGAT	60
TAAACCCTAC TAATTTCTCG TCGGATGCCT CACAACAAGG TGCCTTCCAA GAACTAATGG	120
CCAAAATATC CACCCACAAC ACAAATAAGC TTAGAAAATC TCTTCTTACA ATCCTGACAC	180
AATGGAAGTT TCCCTAAACC ACCCAGCATC TAATACAACC AGCACAAAGA ACAACAACCTC	240
GGCATTTTTT TACTTTGAGT CCTGTCAACC CCCTTCTCCA GCTTTACTCC TATTATGCAT	300
AGCCTATACT GTGGTCTTAA TTGTGGGCCT TTTTGAAAC CTCTCTCTCA TCATCATCAT	360
CTTTAAGAAG CAGAGAAAAG CTCAGAATTT CACCAGCATA CTGATTGCCA ATCTCTCCCT	420
CTCTGATACC TTGGTGTGTG TCATGTGCAT CCATTTTACT ATCATCTACA CTCTGATGGA	480
CCACTGGATA TTTGGGGATA CCATGTGCAG ACTCACATCC TATGTGCAGA GTGTCTCAAT	540
CTCTGTGTCC ATATTCTCAC TTGTATTAC TGCTGTGCGAA AGATATCAGC TAATTGTGAA	600
CCCCCGTGGC TGGAAGCCCA GTGTGACTCA TGCCTACTGG GGCATCACAC TGATTTGGCT	660

GTTTTCCCTT CTGCTGTCTA TTCCCTTCTT CCTGTCCTAC CACCTCACTG ATGAGCCCTT 720  
 CCACAACCTC TCTCTCCCCA CTGACCTCTA CACCCACCAG GTGGCCTGTG TGGAGAAGCTG 780  
 GCCCTCCAAA AAGGACCGGC TGCTCTTCAC CACCTCCCTT TTTCTGCTGC AGTATTTTGT 840  
 TCCTCTAGGC TTCATCCTCA TCTGCTACTT GAAGATTGTT ATCTGCCTCC GCAGGAGAAA 900  
 TGCAAAGGTA GATAAGAAGA AGGAAAATGA GGGCCGGCTC AATGAGAACA AGAGGATCAA 960  
 CACAATGTTG ATTTCCATCG TGGTGACCTT TGGAGCCTGC TGGCTGCCCC CGAATATCTT 1020  
 CAATGTCATC TTTGACTGGT ATCATGAGGT GCTGATGAGC TGCCACCACG ACCTGGTATT 1080  
 TGTAGTTTGC CACTTGGTTG CTATGGTTTC CACATGTATA AACCCCTCTCT TTTATGGCTT 1140  
 TCTCAACAAA AATTTCCAAA AGGACCTGGT AGTGCTTATT CACCACTGCT GGTGCTTCAC 1200  
 ACCTCAGGAA AGATGTGAAA ATATTGCCAT CTCCACTATG CACACAGACT CCAAGAGGTC 1260  
 TTTAAGATTG GCTCGTATAA CAACAGGTAT ATGAAAATTG ATAATGCTGA AGCTCTTCTT 1320  
 GAATGGGAGC TGGACAGGTA ATGGTGGGAA TAGGGCAAGA TGCAGAAAAGA AGAAACCAGA 1380  
 ACCAAAAATA GCAACTTTAT ACCCACTTTT CCTTTAGGCT AAGACTGCCT GTCTCATATG 1440  
 TCTATCCAAC ACACCCTCCG GAATTCGATA TCAAGCTTAT CGATACCGTC GACCTCGAG 1499

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 370 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met	Glu	Val	Ser	Leu	Asn	His	Pro	Ala	Ser	Asn	Thr	Thr	Ser	Thr	Lys
1				5					10					15	
Asn	Asn	Asn	Ser	Ala	Phe	Phe	Tyr	Phe	Glu	Ser	Cys	Gln	Pro	Pro	Ser
			20					25					30		
Pro	Ala	Leu	Leu	Leu	Leu	Cys	Ile	Ala	Tyr	Thr	Val	Val	Leu	Ile	Val
		35					40					45			
Gly	Leu	Phe	Gly	Asn	Leu	Ser	Leu	Ile	Ile	Ile	Ile	Phe	Lys	Lys	Gln
	50					55					60				

- 55 -

Arg Lys Ala Gln Asn Phe Thr Ser Ile Leu Ile Ala Asn Leu Ser Leu  
 65 70 75 80  
 Ser Asp Thr Leu Val Cys Val Met Cys Ile His Phe Thr Ile Ile Tyr  
 85 90 95  
 Thr Leu Met Asp His Trp Ile Phe Gly Asp Thr Met Cys Arg Leu Thr  
 100 105 110  
 Ser Tyr Val Gln Ser Val Ser Ile Ser Val Ser Ile Phe Ser Leu Val  
 115 120 125  
 Phe Thr Ala Val Glu Arg Tyr Gln Leu Ile Val Asn Pro Arg Gly Trp  
 130 135 140  
 Lys Pro Ser Val Thr His Ala Tyr Trp Gly Ile Thr Leu Ile Trp Leu  
 145 150 155 160  
 Phe Ser Leu Leu Leu Ser Ile Pro Phe Phe Leu Ser Tyr His Leu Thr  
 165 170 175  
 Asp Glu Pro Phe His Asn Leu Ser Leu Pro Thr Asp Leu Tyr Thr His  
 180 185 190  
 Gln Val Ala Cys Val Glu Asn Trp Pro Ser Lys Lys Asp Arg Leu Leu  
 195 200 205  
 Phe Thr Thr Ser Leu Phe Leu Leu Gln Tyr Phe Val Pro Leu Gly Phe  
 210 215 220  
 Ile Leu Ile Cys Tyr Leu Lys Ile Val Ile Cys Leu Arg Arg Arg Asn  
 225 230 235 240  
 Ala Lys Val Asp Lys Lys Lys Glu Asn Glu Gly Arg Leu Asn Glu Asn  
 245 250 255  
 Lys Arg Ile Asn Thr Met Leu Ile Ser Ile Val Val Thr Phe Gly Ala  
 260 265 270  
 Cys Trp Leu Pro Pro Asn Ile Phe Asn Val Ile Phe Asp Trp Tyr His  
 275 280 285  
 Glu Val Leu Met Ser Cys His His Asp Leu Val Phe Val Val Cys His  
 290 295 300  
 Leu Val Ala Met Val Ser Thr Cys Ile Asn Pro Leu Phe Tyr Gly Phe  
 305 310 315 320  
 Leu Asn Lys Asn Phe Gln Lys Asp Leu Val Val Leu Ile His His Cys  
 325 330 335  
 Trp Cys Phe Thr Pro Gln Glu Arg Cys Glu Asn Ile Ala Ile Ser Thr  
 340 345 350  
 Met His Thr Asp Ser Lys Arg Ser Leu Arg Leu Ala Arg Ile Thr Thr  
 355 360 365

- 56 -

Gly Ile  
370

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 31 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GATCGAATTC GCCATGGAAG TGCTCACAAA C

31

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 31 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GATCTCTAGA CTATATGCCT GTTGGTATGT G

31

- 57 -

WHAT IS CLAIMED IS:

1. An isolated DNA sequence encoding a neuropeptide Y Y<sub>x</sub> receptor, the DNA sequence having a sequence selected from a  
5 sequence as shown in Figure 3, a sequence of substantial homology to the sequence shown in Figure 3, a fragment of the sequence shown in Figure 3, a fragment of the sequence of substantial homology to the sequence shown in Figure 3, a sequence as shown in Figure 5, a  
10 sequence of substantial homology to the sequence shown in Figure 5, a fragment of the sequence shown in Figure 5 or a fragment of the sequence of substantial homology to the sequence shown in Figure 5.
2. The isolated DNA sequence of Claim 1, wherein the  
15 DNA sequence has a sequence selected from a sequence as shown in Figure 3, a sequence of substantial homology to the sequence shown in Figure 3, a fragment of the sequence shown in Figure 3 or a fragment of the sequence of substantial homology to the sequence shown in Figure 3.
- 20 3. The DNA sequence of Claim 2, which encodes a murine NPY Y<sub>x</sub> receptor.
- 25 4. The DNA sequence of Claim 2, having a sequence selected from the sequence shown in Figure 3, the sequence of substantial homology to the sequence shown in Figure 3 or the fragment of the sequence shown in Figure 3 comprising bases 822 to 1934.
- 30 5. An expression vector containing the DNA sequence of Claim 2.
6. A cell transformed by the expression vector of Claim 5.

- 58 -

7. A method of producing a neuropeptide Y Y<sub>x</sub> receptor, comprising culturing the cell of Claim 6 under conditions which allow the production of the neuropeptide Y Y<sub>x</sub> receptor and optionally recovering the neuropeptide Y Y<sub>x</sub> receptor.
- 5 8. The method of Claim 7, wherein the cell is a mammalian cell.
9. The method of Claim 8, wherein the cell is a COS-7  
10 cell.
10. A neuropeptide Y Y<sub>x</sub> receptor produced by the process of Claim 7.
- 15 11. The neuropeptide Y Y<sub>x</sub> receptor of Claim 10, which is characterized by a pharmacological binding profile with affinities of  $PYY \cong NPY \cong [Leu^{31}Pro^{34}]NPY \cong NPY(2-36) > NPY(13-36)$ .
- 20 12. A neuropeptide Y Y<sub>x</sub> receptor, or a functional derivative thereof, in substantially pure form, which is characterized by a pharmacological binding profile with affinities of  $PYY \cong NPY \cong [Leu^{31}Pro^{34}]NPY \cong NPY(2-36) > NPY(13-36)$ .
- 25 13. Antibody immunologically reactive with the receptor of Claim 12.
14. Isolated RNA encoded by the DNA sequence of Claim 2 or its complementary sequence.
- 30 15. A single-stranded DNA, or fragment thereof, with a sequence complementary to the RNA of Claim 14.
16. The DNA of Claim 15, wherein the DNA, or fragment thereof, modulates the expression of the receptor.

- 59 -

17. The isolated DNA sequence of Claim 1, wherein the DNA sequence has a sequence selected from a sequence as shown in Figure 5, a sequence of substantial homology to the sequence shown in Figure 5, a fragment of the sequence shown in Figure 5 or a fragment of the sequence of substantial homology to the sequence shown in Figure 5.

18. The DNA sequence of Claim 17, which encodes a human NPY Y<sub>x</sub> receptor.

19. The DNA sequence of Claim 17, having a sequence selected from the sequence shown in Figure 5, the sequence of substantial homology to the sequence shown in Figure 5 or the fragment of the sequence shown in Figure 5 comprising bases 182 to 1291.

20. An expression vector containing the DNA sequence of Claim 17.

21. A cell transformed by the expression vector of Claim 20.

22. A method of producing a neuropeptide Y Y<sub>x</sub> receptor, comprising culturing the cell of Claim 21 under conditions which allow the production of the neuropeptide Y Y<sub>x</sub> receptor and optionally recovering the neuropeptide Y Y<sub>x</sub> receptor.

23. The method of Claim 22, wherein the cell is a mammalian cell.

24. The method of Claim 23, wherein the cell is selected from a COS-7 or a CHO cell.

- 60 -

25. A neuropeptide Y Yx receptor produced by the process of Claim 22.

5 26. The neuropeptide Y Yx receptor of Claim 25, which is characterized by a pharmacological binding profile with affinities of  $PYY \cong NPY \cong [Leu^{31}Pro^{34}]NPY \cong NPY(2-36) > NPY(13-36)$ .

10 27. Isolated RNA encoded by the DNA sequence of Claim 17 or its complementary sequence.

28. A single-stranded DNA, or fragment thereof, with a sequence complementary to the RNA of Claim 27.

15 29. The DNA of Claim 28, wherein the DNA, or fragment thereof, modulates the expression of the receptor.

30. A method of identifying compounds that modulate neuropeptide Y Yx receptor activity or modulate binding of ligands to the NPY Yx receptor, comprising:

- 20 (a) mixing a test compound with a solution containing the neuropeptide Y Yx receptor, or functional derivative thereof, to form a mixture;
- (b) measuring NPY Yx receptor activity in the mixture; and
- 25 (c) comparing the receptor activity of the mixture to a standard.

30 31. The method of Claim 30, wherein the neuropeptide Y Yx receptor, or functional derivative thereof, is characterized by a pharmacological binding profile with affinities of  $PYY \cong NPY \cong [Leu^{31}Pro^{34}]NPY \cong NPY(2-36) > NPY(13-36)$ .

32. A method of identifying compounds that modulate the NPY Yx receptor, comprising:

- 61 -

(a) mixing a test compound with cells which express the NPY Yx receptor to form a mixture;

(b) culturing the cells in the presence of the test compound;

5 (c) measuring NPY Yx receptor or second messenger activity in the mixture; and

(d) comparing the NPY Yx receptor activity of the mixture to a standard.

10 33. The method of Claim 32, wherein the cells are transformed by an expression vector containing a sequence selected from a sequence as shown in Figure 3, a sequence of substantial homology to the sequence shown in Figure 3, a fragment of the sequence shown in Figure 3, a fragment of the sequence of substantial  
15 homology to the sequence shown in Figure 3, a sequence as shown in Figure 5, a sequence of substantial homology to the sequence shown in Figure 5, a fragment of the sequence shown in Figure 5 or a fragment of the sequence of substantial homology to the sequence shown in Figure 5.

20 34. The method of Claim 33, wherein the expression vector contains a DNA sequence selected from a DNA sequence having a sequence selected from a sequence as shown in Figure 3, a sequence of substantial homology to the sequence shown in Figure 3, a fragment of  
25 the sequence shown in Figure 3 or a fragment of the sequence of substantial homology to the sequence shown in Figure 3.

30 35. The method of Claim 34, wherein the expression vector contains a DNA sequence selected from the sequence shown in Figure 3, the sequence of substantial homology to the sequence shown in Figure 3 or the fragment of the sequence shown in Figure 3 comprising bases 822 to 1934.

- 62 -

36. The method of Claim 33, wherein the expression vector contains a DNA sequence selected from a DNA sequence having a sequence selected from a sequence as shown in Figure 5, a sequence of substantial homology to the sequence shown in Figure 5, a fragment of the sequence shown in Figure 5 or a fragment of the sequence of substantial homology to the sequence shown in Figure 5.

37. The method of Claim 36, wherein the expression vector contains a DNA sequence selected from the sequence shown in Figure 5, the sequence of substantial homology to the sequence shown in Figure 5 or the fragment of the sequence shown in Figure 5 comprising bases 182 to 1291.

38. A neuropeptide Y Y<sub>x</sub> receptor in substantially pure form comprising an amino acid sequence selected from a sequence as shown in Figure 3 (SEQ. I.D. NO. 6), a sequence as shown in Figure 6 (SEQ. I.D. NO. 12), or a functional derivative thereof.

39. The neuropeptide Y Y<sub>x</sub> receptor of Claim 38, wherein the receptor has an amino acid sequence as shown in Figure 3 (SEQ. I.D. NO. 6) or a functional derivative thereof.

40. The neuropeptide Y Y<sub>x</sub> receptor of Claim 38, wherein the receptor has an amino acid sequence as shown in Figure 6 (SEQ. I.D. NO. 12) or a functional derivative thereof.

41. A method of identifying compounds that bind to a NPY Y<sub>x</sub> receptor comprising:

- a) cloning DNA which encodes a neuropeptide Y Y<sub>x</sub> receptor;
- b) splicing the DNA into an expression vector to produce a construct such that the NPY Y<sub>x</sub> receptor is operably linked to transcription and translation signals sufficient to induce expression of

- 63 -

the NPY Y<sub>x</sub> receptor upon introduction of the construct into a prokaryotic or eukaryotic cell;

5 c) introducing the construct into a prokaryotic or eukaryotic cell which does not express the NPY Y<sub>x</sub> receptor in the absence of the introduced construct; and

10 d) incubating cells or membranes isolated from cells produced in step c with a quantifiable compound known to bind to the NPY Y<sub>x</sub> receptor, and subsequently adding test compounds at a range of concentrations so that the test compounds compete with the quantifiable compound for the NPY Y<sub>x</sub> receptor, such that an IC<sub>50</sub> for the test compound is obtained as the concentration of test compound at which 50% of the quantifiable compound becomes displaced from the NPY Y<sub>x</sub> receptor.

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/01444

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.2, 69.1, 70.1, 70.3, 240.1, 240.2, 320.1; 530/350; 536/23.1, 23.5, 24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	HERZOG et al. Cloned human neuropeptide Y receptor couples to two different second messenger systems. Proc. Natl. Acad. Sci., U.S.A. July 1992, Vol. 89, pages 5794-5798, especially pages 5795-5797.	1-8, 10, 14-25, 27-30, 32-41 ----- 11-12, 26, 31
X ---- Y	WO 93/24515 A1 (CORNELL RESEARCH FOUNDATION, INC.) 09 December 1993, pages 7-13 and 17-31.	1-8, 10, 14-23, 25, 27-30, 32-41 ----- 9
Y	COHEN et al. Characterization of a mouse beta1-adrenergic receptor genomic clone. DNA and Cell Bio. July-August 1993, Vol. 12, pages 537-547, especially page 541.	9

Further documents are listed in the continuation of Box C.  See patent family annex.

* Special categories of cited documents:	*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
*A* document defining the general state of the art which is not considered to be of particular relevance	*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
*E* earlier document published on or after the international filing date	*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
*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)	* & *	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*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

17 APRIL 1996

Date of mailing of the international search report

30 APR 1996

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/01444

**A. CLASSIFICATION OF SUBJECT MATTER:**  
IPC (6):

C07H 21/02, 21/04; C07K 14/705; C12N 5/10, 15/12, 15/63; C12P 21/02; G01N 33/566, 33/567

**A. CLASSIFICATION OF SUBJECT MATTER:**  
US CL :

435/7.2, 69.1, 70.1, 70.3, 240.1, 240.2, 320.1; 530/350; 536/23.1, 23.5, 24.1; 930/10

**B. FIELDS SEARCHED**

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, BIOSIS, MEDLINE, EMBASE, CAPLUS

search terms: neuropeptide, NPY, YY, Y1, Y2, Y3, Y4, PYY, receptor#

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING**

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-12 and 14-41, drawn to nucleic acids, vectors, host cells, methods of producing NPY receptors, the NPY receptors themselves, and a method of using the NPY receptors in receptor binding/second messenger assays.

Group II, claim(s) 13, drawn to an antibody against the NPY receptor.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the antibody of Group II does not share the special technical feature of encoding for an NPY receptor.

Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/01444

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-12, 14-41

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.