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(54) Title: REGULATION OF HUMAN SEROTONIN-LIKE G PROTEIN-COUPLED RECEPTOR

(57) Abstract: Reagents which regulate human serotonin-like G protein-coupled receptor (5-HT-like GPCR) and reagents which bind to human 5-HT-like GPCR gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, COPD, cardiovascular disorders, cancer, urinary disorders, obesity, diabetes, CNS disorders, asthma, and hematological disorders.

REGULATION OF HUMAN SEROTONIN-LIKE G PROTEIN-COUPLED RECEPTOR

This application claims the benefit of and incorporates by reference co-pending provisional application Serial No. 60/264,071 filed January 26, 2001 and Serial No. 60/324,054 filed September 24, 2001.

TECHNICAL FIELD OF THE INVENTION

The invention relates to the area of G-protein coupled receptors. More particularly, it relates to the area of human serotonin-like G protein-coupled receptor and its regulation.

BACKGROUND OF THE INVENTION

G-Protein Coupled Receptors

Many medically significant biological processes are mediated by signal transduction pathways that involve G-proteins (Lefkowitz, *Nature 351*, 353-354, 1991). The family of G-protein coupled receptors (GPCR) includes receptors for hormones, neurotransmitters, growth factors, and viruses. Specific examples of GPCRs include receptors for such diverse agents as dopamine, calcitonin, adrenergic hormones, endothelin, cAMP, adenosine, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsins, endothelial differentiation gene-1, rhodopsins, odorants, cytomegalovirus, G-proteins themselves, effector proteins such as phospholipase C, adenyl cyclase, and phosphodiesterase, and actuator proteins such as protein kinase A and protein kinase C.

GPCRs possess seven conserved membrane-spanning domains connecting at least eight divergent hydrophilic loops. GPCRs (also known as 7TM receptors) have been

characterized as including these seven conserved hydrophobic stretches of about 20 to 30 amino acids, connecting at least eight divergent hydrophilic loops. Most GPCRs have single conserved cysteine residues in each of the first two extracellular loops, which form disulfide bonds that are believed to stabilize functional protein structure. The seven transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6, and TM7. TM3 has been implicated in signal transduction.

Phosphorylation and lipidation (palmitylation or farnesylation) of cysteine residues can influence signal transduction of some GPCRs. Most GPCRs contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several GPCRs, such as the β-adrenergic receptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

For some receptors, the ligand binding sites of GPCRs are believed to comprise hydrophilic sockets formed by several GPCR transmembrane domains. The hydrophilic sockets are surrounded by hydrophobic residues of the GPCRs. The hydrophilic side of each GPCR transmembrane helix is postulated to face inward and form a polar ligand binding site. TM3 has been implicated in several GPCRs as having a ligand binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine, and TM6 or TM7 phenylalanines or tyrosines also are implicated in ligand binding.

GPCRs are coupled inside the cell by heterotrimeric G-proteins to various intracellular enzymes, ion channels, and transporters (*see* Johnson *et al.*, *Endoc. Rev.* *10*, 317-331, 1989). Different G-protein alpha-subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of GPCRs is an important mechanism for the regulation of some GPCRs. For example, in one form of signal transduction, the effect of hormone binding is the activation inside the cell of the enzyme, adenylate cyclase. Enzyme activation by hormones is dependent on the presence of the

nucleotide GTP. GTP also influences hormone binding. A G-protein connects the hormone receptor to adenylate cyclase. G-protein exchanges GTP for bound GDP when activated by a hormone receptor. The GTP-carrying form then binds to activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself, returns the G-protein to its basal, inactive form. Thus, the G-protein serves a dual role, as an intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

Over the past 15 years, nearly 350 therapeutic agents targeting GPCRs receptors have been successfully introduced onto the market. This indicates that these receptors have an established, proven history as therapeutic targets. Clearly, there is an on-going need for identification and characterization of further GPCRs which can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, infections such as bacterial, fungal, protozoan, and viral infections, particularly those caused by HIV viruses, pain, cancers, anorexia, bulimia, asthma, Parkinson's diseases, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, several mental retardation, and dyskinesias, such as Huntington's disease and Tourett's syndrome.

Serotonin

Serotonin is a neuromodulator capable of inducing and modulating a wide variety of behavioral functions such as sleep, appetite, locomotion, sexual activity and vascular contraction. Since the discovery of serotonin (5-hydroxytryptamine, 5-HT) over four decades ago, the cumulative results of many diverse studies have indicated that serotonin plays a significant role in the functioning of the mammalian body, both in the central nervous system and in peripheral systems. Morphological studies of the

central nervous system have shown that serotonergic neurons, which originate in the brain stem, form a very diffuse system that projects to most areas of the brain and spinal cord (O'Brien, Serotonin in Mental Abnormalities 1:41, 1978; Steinbusch, "Handbook of Chemical Neuroanatomy", Volume 3, Part II, 68, 1984; Anden *et al.*, *Acta Physiologica Scandinavia* *67*, 313, 1966). These studies have been complemented by biochemical evidence that indicates large concentrations of 5-HT exists in the brain and spinal cord (Steinbusch, 1984). *See* U.S. Patent 5,698,444.

With such a diffuse system, it is not surprising that 5-HT has been implicated as being involved in the expression of a number of behaviors, physiological responses, and diseases which originate in the central nervous system. These include such diverse areas as sleeping, eating, perceiving pain, controlling body temperature, controlling blood pressure, depression, schizophrenia, and other bodily states (Fuller, Biology of Serotonergic Transmission 21, 1982; Boullin, Serotonin in Mental Abnormalities 1, 316, 1978; Barchas *et al.*, SEROTONIN AND BEHAVIOR, 1973).

Serotonin plays an important role in peripheral systems as well. For example, approximately 90% of the body's serotonin is found in the gastrointestinal system, and serotonin has been found to mediate a variety of contractile, secretory, and electrophysiologic effects in this system. Another example of a peripheral network that is very sensitive to serotonin is the cardiovascular system, which also contains its own source of serotonin, *i.e.*, the platelet.

Given the broad distribution of serotonin within the body, it is understandable that tremendous interest in drugs that affect serotonergic systems exists. In particular, receptor-specific agonists and antagonists are of interest for the treatment of a wide range of disorders, including anxiety, depression, hypertension, migraine, compulsive disorders, schizophrenia, autism, neurodegenerative disorders, such as Alzheimer's disease, Parkinsonism, and Huntington's chorea, and cancer chemotherapy-induced vomiting (Gershon *et al.*, The Peripheral Actions of 5-

Hydroxytryptamine 246, 1989; Saxena *et al.*, *Journal of Cardiovascular Pharmacology 15, Supplement 7*, 990).

Serotonin Receptors

Serotonin produces its effects on cellular physiology by binding to specialized receptors on the cell surface. It is now recognized that multiple types of receptors exist for all neurotransmitters and hormones, including serotonin. The existence of multiple, structurally distinct serotonin receptors has provided the possibility that subtype-selective pharmacologic agents can be produced. The development of such compounds could result in new and increasingly selective therapeutic agents with fewer side effects, since individual receptor subtypes may function to affect specific actions of the different parts of the central peripheral serotonergic systems.

An example of such specificity can be demonstrated by using the vascular system as an example. In certain blood vessels, stimulation of 5-HT_1 -like receptors on the endothelial cells produces vasodilation while stimulation of 5-HT_2 receptors on the smooth muscle cells produces vasoconstriction. Currently, the major classes of serotonin receptors (5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7) contain some fourteen to eighteen separate receptors that have been formally classified based on their pharmacological or structural differences (*see* Glennon *et al.*, *Neuroscience and Behavioral Reviews 14*, 35, 1990).

The 5-HT receptors which have been described to date belong either to the family of receptors associated with ion channels (5-HT_3 receptors), or to the family of receptors which interact with G proteins and which possess seven transmembrane domains. Moreover, analysis of the amino acid sequences has shown that the 5-HT receptors which interact with G proteins may be subdivided into two distinct groups: 5-HT_1 receptors, comprising the mammalian subtypes 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D}, as well as three *Drosophila* 5-HT receptors, and 5-HT_2 receptors comprising the subtypes 5-

HT_2 and 5-HT_{1C}. Pharmacological studies have revealed other subtypes, such as 5-HT_4 receptors, as well as some receptors related to the subtype 5-HT_1 ("5-HT1-like" receptors). Furthermore, additional molecular biology studies have also revealed heterogeneities within the subtypes 5-$HT_{1B}/_{1D}$.

The 5-HT_2 receptors act via phospholipase C, and are responsible for numerous physiological activities of serotonin at the central and peripheral level. At the cardio-vascular level, they are involved in the contraction of the blood vessels and in the morphological changes of the platelets; in the central nervous system, they act on the sensitization of the neurons to tactile stimuli and on the mediation of the hallucinogenic effects of diethylamidelysergic acid, and of the related phenylisopropylamines. Numerous studies show that the 5-HT_2 receptors described up until now do not account for all the properties which are attributed to them. In particular, certain 5-HT_2-types effects of serotonin on the peripheral smooth muscles are classified as atypical effects, of which the effects are thought to be mediated by unknown receptors. *See* U.S. Patent 5,780,245.

Because of serotonin's diverse biological effects, there is a need in the art to identify additional members of the serotonin GCPR family whose activity can be regulated to provide therapeutic effects.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating a human serotonin-like G protein-coupled receptor 1 protein. This and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a serotonin-like G protein-coupled receptor polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 27% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a serotonin-like G protein-coupled receptor polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 27% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

Binding between the test compound and the serotonin-like G protein-coupled receptor polypeptide is detected. A test compound which binds to the serotonin-like G protein-coupled receptor polypeptide is thereby identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the activity of the serotonin-like G protein-coupled receptor.

Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a serotonin-like G protein-coupled receptor polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the serotonin-like G protein-coupled receptor through interacting with the serotonin-like G protein-coupled receptor mRNA.

Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a serotonin-like G protein-coupled receptor polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 27% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

A serotonin-like G protein-coupled receptor activity of the polypeptide is detected. A test compound which increases serotonin-like G protein-coupled receptor activity of the polypeptide relative to serotonin-like G protein-coupled receptor activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation. A test compound which decreases serotonin-like G protein-coupled receptor activity of the polypeptide relative to serotonin-like G protein-coupled receptor activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a

serotonin-like G protein-coupled receptor product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

Binding of the test compound to the serotonin-like G protein-coupled receptor product is detected. A test compound which binds to the serotonin-like G protein-coupled receptor product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a serotonin-like G protein-coupled receptor polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

Serotonin-like G protein-coupled receptor activity in the cell is thereby decreased.

The invention thus provides a human serotonin-like G protein-coupled receptor which can be used to identify test compounds which may act as agonists or antagonists at the receptor site. Human serotonin-like G protein-coupled receptor

and fragments thereof also are useful in raising specific antibodies which can block the receptor and effectively prevent ligand binding.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the DNA-sequence encoding a serotonin-like G protein-coupled receptor Polypeptide (SEQ ID NO:1).

Fig. 2 shows the amino acid sequence deduced from the DNA-sequence of Fig.1 (SEQ ID NO:2).

Fig. 3 shows the amino acid sequence of the protein identified by SwissProt Accession No. P28222|5H1B_HUMAB5-HYDROXYTRYPTAMINE 1B RECEPTOR (5-HT-1B) (SEROTONIN RECEPTOR) (SEQ ID NO:3).

Fig. 4 shows the DNA-sequence encoding a serotonin-like G protein-coupled receptor Polypeptide (SEQ ID NO:4).

Fig. 5 shows the BLASTP - alignment of 414 (SEQ ID NO:2) against swiss|P28222|5H1B_HUMAN5-HYDROXYTRYPTAMINE 1B RECEPTOR (5-HT-1B) (SEROTONIN RECEPTOR) (SEQ ID NO:3).

Fig. 6 shows the HMMPFAM - alignment of 414 (SEQ ID NO:2) against pfam|hmm|7tm_17 transmembrane receptor (rhodopsin family).

Fig. 7 shows the Genescan results.

Fig. 8 shows the Predicted amino acid and nucleotide sequences.

Fig. 9 shows the BLASTN - alignment of 414_DNA against SNP:1275729_867778.

Fig. 10 shows the Transmembrane regions of SEQ ID NO:2.

Fig. 11 shows the Expression profiles.

Fig. 12 shows the Expression profiles.

Fig. 13 shows the Expression profiles

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated polynucleotide being selected from the group consisting of:

a) a polynucleotide encoding a serotonin-like G protein-coupled receptor polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 27% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

b) a polynucleotide comprising the sequence of SEQ ID NO: 1;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b) and encodes a serotonin-like G protein-coupled receptor polypeptide;

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes a serotonin-like G protein-coupled receptor polypeptide; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and encodes a serotonin-like G protein-coupled receptor polypeptide.

Furthermore, it has been discovered by the present applicant that a novel 5-HT-like GPCR, particularly a human 5-HT-like GPCR, can be used in therapeutic methods to treat COPD, cardiovascular disorders, cancer, urinary disorders, obesity, diabetes, CNS disorders, asthma, or hematological disorders. Human 5-HT-like GPCR comprises the amino acid sequence shown in SEQ ID NO:2. A coding sequence for human 5-HT-like GPCR is shown in SEQ ID NO:1. This sequence is contained within the longer sequence shown in SEQ ID NO:4. This sequence is located on chromosome 2 and was assembled from the genomic sequence of Accession No. AC060810 using Genescan or Geneid.

Human 5-HT-like GPCR is 26% identical over 272 amino acids to swiss|P28222|5H1B_HUMAN5-HYDROXYTRYPTAMINE 1B RECEPTOR (5-HT-1B) (SEROTONIN RECEPTOR) (SEQ ID NO:3) (Fig. 5). Pfam detected seven transmembrane motifs, which are shown in Fig. 10.

Human 5-HT-like GPCR of the invention is expected to be useful for the same purposes as previously identified serotonin receptors. Human 5-HT-like GPCR is believed to be useful in therapeutic methods to treat disorders such as COPD, cardiovascular disorders, cancer, urinary disorders, obesity, diabetes, CNS disorders, asthma, and hematological disorders. Human 5-HT-like GPCR also can be used to screen for human 5-HT-like GPCR activators and inhibitors.

5-HT-like GPCR Polypeptides

5-HT-like GPCR polypeptides according to the invention comprise at least 6, 8 10, 12, 15, 18, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, or 347 contiguous amino acids selected from SEQ ID NO:2 or from a biologically active variant thereof, as defined below. A 5-HT-like GPCR polypeptide of the invention therefore can be a portion of a 5-HT-like GPCR protein, a full-length 5-HT-like GPCR protein, or a fusion protein comprising all or a portion of a 5-HT-like GPCR protein. A coding sequence for SEQ ID NO:2 is shown in SEQ ID NO:1.

Biologically Active Variants

5-HT-like GPCR polypeptide variants which are biologically active, *i.e.*, retain the ability to bind a serotonin or a serotonin-like ligand to produce a biological effect, such as cyclic AMP formation, mobilization of intracellular calcium, or phospho-inositide metabolism, also are 5-HT-like GPCR polypeptides. Preferably, naturally or non-naturally occurring 5-HT-like GPCR polypeptide variants have amino acid

sequences which are at least about 27, 30, 35, 40, 45, 50, 55, 60, 65, 70, preferably about 75, 80, 85, 90, 95, 96, 97, 98, 0r 99% identical to the amino acid sequence shown in SEQ ID NO:2 or a fragment thereof. Percent identity between a putative 5-HT-like GPCR polypeptide variant and an amino acid sequence of SEQ ID NO:2 is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48:603 (1986), and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM62" scoring matrix of Henikoff and Henikoff (ibid.).Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA"similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative variant. The FASTA algorithm is described y Pearson and Lipman, Proc. Nat'l Acad. Sci. USA 85:2444(1988), and by Pearson, Meth. Enzymol. 183:63 (1990).Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (e.g. SEQ ID NO:2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to for man approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch- Sellers algorithm (Needleman and Wunsch, J. Mol. Biol.48:444 (1970); Sellers, SIAM J. Appl. Math. 26:787 (1974)), which allows for amino acid insertions and deletions. Preferred

parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, Meth. Enzymol. 183:63 (1990). FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as default..

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a 5-HT-like GPCR polypeptide can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active 5-HT-like GPCR polypeptide can readily be determined by assaying for binding to a ligand or by conducting a functional assay, as described for example, in the specific Examples, below.

Fusion Proteins

Fusion proteins are useful for generating antibodies against 5-HT-like GPCR polypeptide amino acid sequences and for use in various assay systems. For

example, fusion proteins can be used to identify proteins which interact with portions of a 5-HT-like GPCR polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

A 5-HT-like GPCR polypeptide fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment comprises at 6, 8 10, 12, 15, 18, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, or 347 contiguous amino acids of SEQ ID NO:2 or from a biologically active variant, such as those described above. The first polypeptide segment also can comprise full-length 5-HT-like GPCR protein.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include β-galactosidase, β-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the 5-HT-like GPCR polypeptide-encoding sequence and the heterologous protein sequence, so that the 5-HT-like GPCR polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by

standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from SEQ ID NO:1 in proper reading frame with nucleotides encoding the second polypeptide segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

Species homologs of human 5-HT-like GPCR polypeptide can be obtained using 5-HT-like GPCR polypeptide polynucleotides (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of 5-HT-like GPCR polypeptide, and expressing the cDNAs as is known in the art.

5-HT-like GPCR Polynucleotides

A 5-HT-like GPCR polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a 5-HT-like GPCR polypeptide. A coding sequences for human 5-HT-like GPCR is shown in SEQ ID NO:3.

Degenerate nucleotide sequences encoding human 5-HT-like GPCR polypeptides, as well as homologous nucleotide sequences which are at least about 50, preferably about 75, 90, 96, or 98% identical to the nucleotide sequence shown in SEQ ID NO:1 also are 5-HT-like GPCR polynucleotides. Percent sequence identity between the

sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of 5-HT-like GPCR polynucleotides which encode biologically active 5-HT-like GPCR polypeptides also are 5-HT-like GPCR polynucleotides. Polynucleotides comprising at least 6, 7, 8, 9, 10, 12, 15, 18, 20, or 25 contiguous nucleotides of SEQ ID NO:1 or its complement also are 5-HT-like GPCR polynucleotides. Such polynucleotides can be used, for example, as hybridization probes or antisense oligonucleotides.

Identification of Variants and Homologs of 5-HT-like GPCR Polynucleotides

Variants and homologs of the 5-HT-like GPCR polynucleotides described above also are 5-HT-like GPCR polynucleotides. Typically, homologous 5-HT-like GPCR polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known 5-HT-like GPCR polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50 °C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the 5-HT-like GPCR polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of 5-HT-like GPCR polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5 °C with every 1% decrease in homology (Bonner *et al.*, *J. Mol.*

Biol. 81, 123 (1973). Variants of human 5-HT-like GPCR polynucleotides or 5-HT-like GPCR polynucleotides of other species can therefore be identified by hybridizing a putative homologous 5-HT-like GPCR polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO:1 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising 5-HT-like GPCR polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to 5-HT-like GPCR polynucleotides or their complements following stringent hybridization and/or wash conditions also are 5-HT-like GPCR polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20 °C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a 5-HT-like GPCR polynucleotide having a nucleotide sequence shown in SEQ ID NO:3 or the complement thereof and a polynucleotide sequence which is at least about 50, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A. 48*, 1390 (1962):

$$T_m = 81.5\ ^\circ C - 16.6(\log_{10}[Na^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l),$$

where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65 °C, or 50% formamide, 4X SSC at 42 °C, or 0.5X SSC, 0.1% SDS at 65 °C. Highly stringent wash conditions include, for example, 0.2X SSC at 65 °C.

Preparation of 5-HT-like GPCR Polynucleotides

A naturally occurring 5-HT-like GPCR polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated 5-HT-like GPCR polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprises 5-HT-like GPCR nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

5-HT-like GPCR cDNA molecules can be made with standard molecular biology techniques, using 5-HT-like GPCR mRNA as a template. 5-HT-like GPCR cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesizes 5-HT-like GPCR polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a 5-HT-like GPCR polypeptide having, for example, an amino acid sequence shown in SEQ ID NO:2 or a biologically active variant thereof.

Extending 5-HT-like GPCR Polynucleotides

Various PCR-based methods can be used to extend the nucleic acid sequences disclosed herein to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic. 2*, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res. 16*, 8186, 1988). Primers can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72 °C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic. 1*, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 3055-3060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (*e.g.* GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Obtaining 5-HT-like GPCR Polypeptides

5-HT-like GPCR polypeptides can be obtained, for example, by purification from human cells, by expression of 5-HT-like GPCR polynucleotides, or by direct chemical synthesis.

Protein Purification

5-HT-like GPCR polypeptides can be purified from any human cell which expresses the receptor, including host cells which have been transfected with 5-HT-like GPCR polynucleotides. A purified 5-HT-like GPCR polypeptide is separated from other compounds which normally associate with the 5-HT-like GPCR polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis.

5-HT-like GPCR polypeptide can be conveniently isolated as a complex with its associated G protein, as described in the specific examples, below. A preparation of purified 5-HT-like GPCR polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

Expression of 5-HT-like GPCR Polynucleotides

To express a 5-HT-like GPCR polypeptide, a 5-HT-like GPCR polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding 5-HT-like GPCR polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding a 5-HT-like GPCR polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO, and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a 5-HT-like GPCR polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the 5-HT-like GPCR polypeptide. For example, when a large quantity of a 5-HT-like GPCR polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUE-SCRIPT vector, a sequence encoding the 5-HT-like GPCR polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem. 264*, 5503-5509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol. 153*, 516-544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding 5-HT-like GPCR polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in

combination with the omega leader sequence from TMV (Takamatsu, *EMBO J. 6*, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J. 3*, 1671-1680, 1984; Broglie *et al.*, *Science 224*, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ. 17*, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (*e.g.*, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express a 5-HT-like GPCR polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding 5-HT-like GPCR polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of 5-HT-like GPCR polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda cells* or *Trichoplusia* larvae in which 5-HT-like GPCR polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci. 91*, 3224-3227, 1994).

Mammalian Expression Systems

A number of viral-based expression systems can be used to express 5-HT-like GPCR polypeptides in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding 5-HT-like GPCR polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing a 5-

HT-like GPCR polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci. 81*, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (*e.g.*, liposomes, polycationic amino polymers, or vesicles).

Specific initiation signals also can be used to achieve more efficient translation of sequences encoding 5-HT-like GPCR polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a 5-HT-like GPCR polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ. 20*, 125-162, 1994).

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed 5-HT-like GPCR polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and

acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express 5-HT-like GPCR polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced 5-HT-like GPCR sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

Any number of selection systems can be used to recover transformed cell lines.

These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*, 817-23, 1980) genes which can be employed in *tk*$^-$ or *aprt*$^-$ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci. 77*, 3567-70, 1980), *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol. 150*, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin

acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. 85*, 8047-51, 1988). Visible markers such as anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol. 55*, 121-131, 1995).

Detecting Expression of 5-HT-like GPCR Polypeptides

Although the presence of marker gene expression suggests that the 5-HT-like GPCR polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a 5-HT-like GPCR polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode a 5-HT-like GPCR polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a 5-HT-like GPCR polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the 5-HT-like GPCR polynucleotide.

Alternatively, host cells which contain a 5-HT-like GPCR polynucleotide and which express a 5-HT-like GPCR polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding a 5-HT-like GPCR polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding a 5-HT-like GPCR polypeptide.

Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a 5-HT-like GPCR polypeptide to detect transformants which contain a 5-HT-like GPCR polynucleotide.

A variety of protocols for detecting and measuring the expression of a 5-HT-like GPCR polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a 5-HT-like GPCR polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med. 158*, 1211-1216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding 5-HT-like GPCR polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a 5-HT-like GPCR polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Expression and Purification of 5-HT-like GPCR Polypeptides

Host cells transformed with nucleotide sequences encoding a 5-HT-like GPCR polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode 5-HT-like GPCR polypeptides can be designed to contain signal sequences which direct secretion of soluble 5-HT-like GPCR polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound 5-HT-like GPCR polypeptide.

As discussed above, other constructions can be used to join a sequence encoding a 5-HT-like GPCR polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the 5-HT-like GPCR polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a 5-HT-like GPCR polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif. 3*, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the 5-HT-like GPCR polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol. 12*, 441-453, 1993.

Chemical Synthesis

Sequences encoding a 5-HT-like GPCR polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al. Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a 5-HT-like GPCR polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc.* *85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of 5-HT-like GPCR polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic 5-HT-like GPCR polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the 5-HT-like GPCR polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Production of Altered 5-HT-like GPCR Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce 5-HT-like GPCR polypeptide-encoding nucleotide sequences possessing non-natural-

ly occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter 5-HT-like GPCR polypeptide-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of a 5-HT-like GPCR polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv, which are capable of binding an epitope of a 5-HT-like GPCR polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

An antibody which specifically binds to an epitope of a 5-HT-like GPCR polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous

protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen.

Typically, an antibody which specifically binds to a 5-HT-like GPCR polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to 5-HT-like GPCR polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate a 5-HT-like GPCR polypeptide from solution.

5-HT-like GPCR polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a 5-HT-like GPCR polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

Monoclonal antibodies which specifically bind to a 5-HT-like GPCR polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature 256*, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods 81*, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci. 80*, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol. 62*, 109-120, 1984).

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*, 6851-6855, 1984; Neuberger *et al.*, *Nature 312*, 604-608, 1984; Takeda *et al.*, *Nature 314*, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to a 5-HT-like GPCR polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to 5-HT-like GPCR polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci. 88*, 11120-23, 1991).

Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev. 5*, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol. 15*,

159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem. 269*, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer 61*, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth. 165*, 81-91).

Antibodies which specifically bind to 5-HT-like GPCR polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci. 86*, 3833-3837, 1989; Winter *et al.*, *Nature 349*, 293-299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a 5-HT-like GPCR polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of 5-HT-like GPCR gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. *See* Brown, *Meth. Mol. Biol. 20*, 1-8, 1994; Sonveaux, *Meth. Mol. Biol. 26*, 1-72, 1994; Uhlmann *et al.*, *Chem. Rev. 90*, 543-583, 1990.

Modifications of 5-HT-like GPCR gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of the 5-HT-like GPCR gene. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described

in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a 5-HT-like GPCR polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a 5-HT-like GPCR polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent 5-HT-like GPCR nucleotides, can provide sufficient targeting specificity for 5-HT-like GPCR mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular 5-HT-like GPCR polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a 5-HT-like GPCR polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g.*, Agrawal *et al.*, *Trends Biotechnol. 10*, 152-158; 1992; Uhlmann *et al.*,

Chem. Rev. 90, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett. 215*, 3539-3542, 1987.

Ribozymes

Ribozymes are RNA molecules with catalytic activity. *See, e.g.*, Cech, *Science 236*, 1532-1539; 1987; Cech, *Ann. Rev. Biochem. 59*, 543-568; 1990, Cech, *Curr. Opin. Struct. Biol. 2*, 605-609; 1992, Couture & Stinchcomb, *Trends Genet. 12*, 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g.*, Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a 5-HT-like GPCR polynucleotide, such as the nucleotide sequence shown in SEQ ID NO:3, can be used to generate ribozymes which will specifically bind to mRNA transcribed from the 5-HT-like GPCR polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (*see* Haseloff *et al. Nature 334*, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within a 5-HT-like GPCR RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA

sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate 5-HT-like GPCR RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. The nucleotide sequence shown in SEQ ID NO:3 and its complement provide a source of suitable hybridization region sequences. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease 5-HT-like GPCR expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Differentially Expressed Genes

Described herein are methods for the identification of genes whose products interact with human 5-HT-like GPCR. Such genes may represent genes that are differentially expressed in disorders including, but not limited to, COPD, cardiovascular disorders, cancer, urinary disorders, obesity, diabetes, CNS disorders, asthma, and hematological disorders. Further, such genes may represent genes that are differentially regulated in response to manipulations relevant to the progression or treatment of such diseases. Additionally, such genes may have a temporally modulated expression, increased or decreased at different stages of tissue or organism development. A differentially expressed gene may also have its expression modulated under control versus experimental conditions. In addition, the human 5-HT-like GPCR gene or gene product may itself be tested for differential expression.

The degree to which expression differs in a normal versus a diseased state need only be large enough to be visualized via standard characterization techniques such as differential display techniques. Other such standard characterization techniques by which expression differences may be visualized include but are not limited to, quantitative RT (reverse transcriptase), PCR, and Northern analysis.

Identification of Differentially Expressed Genes

To identify differentially expressed genes total RNA or, preferably, mRNA is isolated from tissues of interest. For example, RNA samples are obtained from tissues of experimental subjects and from corresponding tissues of control subjects. Any RNA isolation technique that does not select against the isolation of mRNA may be utilized for the purification of such RNA samples. See, for example, Ausubel *et al.*, ed., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc. New York, 1987-1993. Large numbers of tissue samples may readily be processed using

techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, U.S. Patent 4,843,155.

Transcripts within the collected RNA samples that represent RNA produced by differentially expressed genes are identified by methods well known to those of skill in the art. They include, for example, differential screening (Tedder *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 85*, 208-12, 1988), subtractive hybridization (Hedrick *et al.*, *Nature 308*, 149-53; Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 88*, 2825, 1984), and differential display (Liang & Pardee, *Science 257*, 967-71, 1992; U.S. Patent 5,262,311), and microarrays.

The differential expression information may itself suggest relevant methods for the treatment of disorders involving the human 5-HT-like GPCR. For example, treatment may include a modulation of expression of the differentially expressed genes and/or the gene encoding the human 5-HT-like GPCR. The differential expression information may indicate whether the expression or activity of the differentially expressed gene or gene product or the human 5-HT-like GPCR gene or gene product are up-regulated or down-regulated.

Screening Methods

The invention provides assays for screening test compounds which bind to or modulate the activity of a 5-HT-like GPCR polypeptide or a 5-HT-like GPCR polynucleotide. A test compound preferably binds to a 5-HT-like GPCR polypeptide or polynucleotide. More preferably, a test compound decreases or increases the effect of serotonin or a serotonin analog as mediated via human 5-HT-like GPCR by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. *See* Lam, *Anticancer Drug Des. 12*, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (*see*, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 90*, 6909, 1993; Erb *et al. Proc. Natl. Acad. Sci. U.S.A. 91*, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem. 37*, 2678, 1994; Cho *et al.*, *Science 261*, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2061; Gallop *et al.*, *J. Med. Chem. 37*, 1233, 1994). Libraries of compounds can be presented in solution (*see*, *e.g.*, Houghten, *BioTechniques 13*, 412-421, 1992), or on beads (Lam, *Nature 354*, 82-84, 1991), chips (Fodor, *Nature 364*, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 89*, 1865-1869, 1992), or phage (Scott & Smith, *Science 249*, 386-390, 1990; Devlin, *Science 249*, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci. 97*, 6378-6382, 1990; Felici, *J. Mol. Biol. 222*, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

High Throughput Screening

Test compounds can be screened for the ability to bind to 5-HT-like GPCR polypeptides or polynucleotides or to affect 5-HT-like GPCR activity or 5-HT-like GPCR gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 µl. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 19,* 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the com-

pounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity 2*, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

Binding Assays

For binding assays, the test compound is preferably a small molecule which binds to and occupies the active site of the 5-HT-like GPCR polypeptide, thereby making the ligand binding site inaccessible to substrate such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules. Potential ligands which bind to a polypeptide of the invention include, but are not limited to, the natural ligands of known 5-HT-like GPCRs and analogues or derivatives thereof.

In binding assays, either the test compound or the 5-HT-like GPCR polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the 5-HT-like GPCR polypeptide can then be accomplished, for example, by direct counting of radio-

emmission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

Alternatively, binding of a test compound to a 5-HT-like GPCR polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with a 5-HT-like GPCR polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and a 5-HT-like GPCR polypeptide (McConnell *et al.*, *Science 257*, 1906-1912, 1992).

Determining the ability of a test compound to bind to a 5-HT-like GPCR polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem. 63*, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol. 5*, 699-705, 1995). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, a 5-HT-like GPCR polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell 72*, 223-232, 1993; Madura *et al.*, *J. Biol. Chem. 268*, 12046-12054, 1993; Bartel *et al.*, *BioTechniques 14*, 920-924, 1993; Iwabuchi *et al.*, *Oncogene 8*, 1693-1696, 1993; and Brent W094/10300), to identify other proteins which bind to or interact with the 5-HT-like GPCR polypeptide and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding a 5-HT-like GPCR polypeptide can be fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with the 5-HT-like GPCR polypeptide.

It may be desirable to immobilize either the 5-HT-like GPCR polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the 5-HT-like GPCR polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the 5-HT-like GPCR polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to a 5-HT-like GPCR polypeptide (or

polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, the 5-HT-like GPCR polypeptide is a fusion protein comprising a domain that allows the 5-HT-like GPCR polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed 5-HT-like GPCR polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a 5-HT-like GPCR polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated 5-HT-like GPCR polypeptides (or polynucleotides) or test compounds can be prepared from biotin-NHS(N-hydroxysuccinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a 5-HT-like GPCR polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the active site of the 5-HT-like GPCR polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the 5-HT-like GPCR polypeptide or test compound, enzyme-linked assays which rely on detecting an activity of the 5-HT-like GPCR polypeptide, and SDS gel electrophoresis under non-reducing conditions.

Screening for test compounds which bind to a 5-HT-like GPCR polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a 5-HT-like GPCR polypeptide or polynucleotide can be used in a cell-based assay system. A 5-HT-like GPCR polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the test compound to a 5-HT-like GPCR polypeptide or polynucleotide is determined as described above.

Functional Assays

Test compounds can be tested for the ability to increase or decrease a biological effect of a 5-HT-like GPCR polypeptide. Such biological effects can be determined using the functional assays described in the specific examples, below. Functional assays can be carried out after contacting either a purified 5-HT-like GPCR polypeptide, a cell membrane preparation, or an intact cell with a test compound. A test compound which decreases a functional activity of a 5-HT-like GPCR by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for decreasing 5-HT-like GPCR activity. A test compound which increases 5-HT-like GPCR activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for increasing 5-HT-like GPCR activity.

One such screening procedure involves the use of melanophores which are transfected to express a 5-HT-like GPCR polypeptide. Such a screening technique is

described in WO 92/01810 published Feb. 6, 1992. Thus, for example, such an assay may be employed for screening for a compound which inhibits activation of the receptor polypeptide by contacting the melanophore cells which comprise the receptor with both the receptor ligand (*e.g.*, serotonin or a serotonin analog) and a test compound to be screened. Inhibition of the signal generated by the ligand indicates that a test compound is a potential antagonist for the receptor, *i.e.*, inhibits activation of the receptor. The screen may be employed for identifying a test compound which activates the receptor by contacting such cells with compounds to be screened and determining whether each test compound generates a signal, *i.e.*, activates the receptor.

Other screening techniques include the use of cells which express a human 5-HT-like GPCR polypeptide (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation (*see, e.g., Science 246*, 181-296, 1989). For example, test compounds may be contacted with a cell which expresses a human 5-HT-like GPCR polypeptide and a second messenger response, *e.g.*, signal transduction or pH changes, can be measured to determine whether the test compound activates or inhibits the receptor.

Another such screening technique involves introducing RNA encoding a human 5-HT-like GPCR polypeptide into *Xenopus* oocytes to transiently express the receptor. The transfected oocytes can then be contacted with the receptor ligand and a test compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for test compounds which are thought to inhibit activation of the receptor.

Another screening technique involves expressing a human 5-HT-like GPCR polypeptide in cells in which the receptor is linked to a phospholipase C or D. Such cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The

screening may be accomplished as described above by quantifying the degree of activation of the receptor from changes in the phospholipase activity.

Details of functional assays such as those described above are provided in the specific examples, below.

5-HT-like GPCR Gene Expression

In another embodiment, test compounds which increase or decrease 5-HT-like GPCR gene expression are identified. A 5-HT-like GPCR polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the 5-HT-like GPCR polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of 5-HT-like GPCR mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of a 5-HT-like GPCR polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro*

translation system by detecting incorporation of labeled amino acids into a 5-HT-like GPCR polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses a 5-HT-like GPCR polynucleotide can be used in a cell-based assay system. The 5-HT-like GPCR polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, such as CHO or human embryonic kidney 293 cells, can be used.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions which can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, a 5-HT-like GPCR polypeptide, 5-HT-like GPCR polynucleotide, antibodies which specifically bind to a 5-HT-like GPCR polypeptide, or mimetics, agonists, antagonists, or inhibitors of a 5-HT-like GPCR polypeptide activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal,

subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxy-propylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be

dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

Therapeutic Indications and Methods

GPCRs are ubiquitous in the mammalian host and are responsible for many biological functions, including many pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate a GPCR on the one hand and which can inhibit the function of a GPCR on the other hand. For example, compounds which activate a GPCR may be employed for therapeutic purposes, such as the treatment of asthma, Parkinson's disease, acute heart failure, urinary retention, and osteoporosis. In particular, compounds which activate GPCRs are useful in treating various cardiovascular ailments such as caused by the lack of pulmonary blood flow or hypertension. In addition these compounds may also be used in treating various physiological disorders relating to abnormal control of fluid and electrolyte homeostasis and in diseases associated with abnormal angiotensin-induced aldosterone secretion.

In general, compounds which inhibit activation of a GPCR can be used for a variety of therapeutic purposes, for example, for the treatment of hypotension and/or hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders including schizophrenia, manic excitement, depression, delirium, dementia or severe mental retardation, dyskinesias, such as Huntington's disease or Tourett's syndrome, among others. Compounds which inhibit GPCRs also are useful in reversing endogenous anorexia, in the control of bulimia, and in treating various cardiovascular ailments

such as caused by excessive pulmonary blood flow or hypotension. In particular, regulation of 5-HT-like GPCR can be used to treat anxiety, depression, hypertension, migraine, compulsive disorders, schizophrenia, autism, neurodegenerative disorders, such as Alzheimer's disease, Parkinsonism, and Huntington's chorea, and cancer chemotherapy-induced vomiting, as well as sleep and eating disorders, pain control, disorders involving regulation of body temperature and blood pressure.

Diabetes

Human 5-HT-like GPCR can be regulated to treat diabetes. Diabetes mellitus is a common metabolic disorder characterized by an abnormal elevation in blood glucose, alterations in lipids and abnormalities (complications) in the cardiovascular system, eye, kidney and nervous system. Diabetes is divided into two separate diseases: type 1 diabetes (juvenile onset), which results from a loss of cells which make and secrete insulin, and type 2 diabetes (adult onset), which is caused by a defect in insulin secretion and a defect in insulin action.

Type 1 diabetes is initiated by an autoimmune reaction that attacks the insulin secreting cells (beta cells) in the pancreatic islets. Agents that prevent this reaction from occurring or that stop the reaction before destruction of the beta cells has been accomplished are potential therapies for this disease. Other agents that induce beta cell proliferation and regeneration also are potential therapies.

Type II diabetes is the most common of the two diabetic conditions (6% of the population). The defect in insulin secretion is an important cause of the diabetic condition and results from an inability of the beta cell to properly detect and respond to rises in blood glucose levels with insulin release. Therapies that increase the response by the beta cell to glucose would offer an important new treatment for this disease.

The defect in insulin action in Type II diabetic subjects is another target for therapeutic intervention. Agents that increase the activity of the insulin receptor in muscle, liver, and fat will cause a decrease in blood glucose and a normalization of plasma lipids. The receptor activity can be increased by agents that directly stimulate the receptor or that increase the intracellular signals from the receptor. Other therapies can directly activate the cellular end process, *i.e.* glucose transport or various enzyme systems, to generate an insulin-like effect and therefore a produce beneficial outcome. Because overweight subjects have a greater susceptibility to Type II diabetes, any agent that reduces body weight is a possible therapy.

Both Type I and Type diabetes can be treated with agents that mimic insulin action or that treat diabetic complications by reducing blood glucose levels. Likewise, agents that reduces new blood vessel growth can be used to treat the eye complications that develop in both diseases.

Obesity

Obesity and overweight are defined as an excess of body fat relative to lean body mass. An increase in caloric intake or a decrease in energy expenditure or both can bring about this imbalance leading to surplus energy being stored as fat. Obesity is associated with important medical morbidities and an increase in mortality. The causes of obesity are poorly understood and may be due to genetic factors, environmental factors or a combination of the two to cause a positive energy balance. In contrast, anorexia and cachexia are characterized by an imbalance in energy intake versus energy expenditure leading to a negative energy balance and weight loss. Agents that either increase energy expenditure and/or decrease energy intake, absorption or storage would be useful for treating obesity, overweight, and associated comorbidities. Agents that either increase energy intake and/or decrease energy expenditure or increase the amount of lean tissue would be useful for treating cachexia, anorexia and wasting disorders.

This gene, translated proteins and agents which modulate this gene or portions of the gene or its products are useful for treating obesity, overweight, anorexia, cachexia, wasting disorders, appetite suppression, appetite enhancement, increases or decreases in satiety, modulation of body weight, and/or other eating disorders such as bulimia. Also this gene, translated proteins and agents which modulate this gene or portions of the gene or its products are useful for treating obesity/overweight-associated comorbidities including hypertension, type 2 diabetes, coronary artery disease, hyperlipidemia, stroke, gallbladder disease, gout, osteoarthritis, sleep apnea and respiratory problems, some types of cancer including endometrial, breast, prostate, and colon cancer, thrombolic disease, polycystic ovarian syndrome, reduced fertility, complications of pregnancy, menstrual irregularities, hirsutism, stress incontinence, and depression.

CNS disorders

CNS disorders which may be treated include brain injuries, cerebrovascular diseases and their consequences, Parkinson's disease, corticobasal degeneration, motor neuron disease, dementia, including ALS, multiple sclerosis, traumatic brain injury, stroke, post-stroke, post-traumatic brain injury, and small-vessel cerebrovascular disease. Dementias, such as Alzheimer's disease, vascular dementia, dementia with Lewy bodies, frontotemporal dementia and Parkinsonism linked to chromosome 17, frontotemporal dementias, including Pick's disease, progressive nuclear palsy, corticobasal degeneration, Huntington's disease, thalamic degeneration, Creutzfeld-Jakob dementia, HIV dementia, schizophrenia with dementia, and Korsakoff's psychosis also can be treated. Similarly, it may be possible to treat cognitive-related disorders, such as mild cognitive impairment, age-associated memory impairment, age-related cognitive decline, vascular cognitive impairment, attention deficit disorders, attention deficit hyperactivity disorders, and memory disturbances in

children with learning disabilities, by regulating the activity of human 5HT-like GPCR.

Pain that is associated with CNS disorders also can be treated by regulating the activity of human 5HT-like GPCR. Pain which can be treated includes that associated with central nervous system disorders, such as multiple sclerosis, spinal cord injury, sciatica, failed back surgery syndrome, traumatic brain injury, epilepsy, Parkinson's disease, post-stroke, and vascular lesions in the brain and spinal cord (*e.g.*, infarct, hemorrhage, vascular malformation). Non-central neuropathic pain includes that associated with post mastectomy pain, reflex sympathetic dystrophy (RSD), trigeminal neuralgiaradioculopathy, post-surgical pain, HIV/AIDS related pain, cancer pain, metabolic neuropathies (*e.g.*, diabetic neuropathy, vasculitic neuropathy secondary to connective tissue disease), paraneoplastic polyneuropathy associated, for example, with carcinoma of lung, or leukemia, or lymphoma, or carcinoma of prostate, colon or stomach, trigeminal neuralgia, cranial neuralgias, and post-herpetic neuralgia. Pain associated with cancer and cancer treatment also can be treated, as can headache pain (for example, migraine with aura, migraine without aura, and other migraine disorders), episodic and chronic tension-type headache, tension-type like headache, cluster headache, and chronic paroxysmal hemicrania.

Cardiovascular disorders

Cardiovascular diseases include the following disorders of the heart and the vascular system: congestive heart failure, myocardial infarction, ischemic diseases of the heart, all kinds of atrial and ventricular arrhythmias, hypertensive vascular diseases, and peripheral vascular diseases.

Heart failure is defined as a pathophysiologic state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate

commensurate with the requirement of the metabolizing tissue. It includes all forms of pumping failure, such as high-output and low-output, acute and chronic, right-sided or left-sided, systolic or diastolic, independent of the underlying cause.

Myocardial infarction (MI) is generally caused by an abrupt decrease in coronary blood flow that follows a thrombotic occlusion of a coronary artery previously narrowed by arteriosclerosis. MI prophylaxis (primary and secondary prevention) is included, as well as the acute treatment of MI and the prevention of complications.

Ischemic diseases are conditions in which the coronary flow is restricted resulting in a perfusion which is inadequate to meet the myocardial requirement for oxygen. This group of diseases includes stable angina, unstable angina, and asymptomatic ischemia.

Arrhythmias include all forms of atrial and ventricular tachyarrhythmias (atrial tachycardia, atrial flutter, atrial fibrillation, atrio-ventricular reentrant tachycardia, preexcitation syndrome, ventricular tachycardia, ventricular flutter, and ventricular fibrillation), as well as bradycardic forms of arrhythmias.

Vascular diseases include primary as well as all kinds of secondary arterial hypertension (renal, endocrine, neurogenic, others). The disclosed gene and its product may be used as drug targets for the treatment of hypertension as well as for the prevention of all complications.

Peripheral vascular diseases are defined as vascular diseases in which arterial and/or venous flow is reduced resulting in an imbalance between blood supply and tissue oxygen demand. It includes chronic peripheral arterial occlusive disease (PAOD), acute arterial thrombosis and embolism, inflammatory vascular disorders, Raynaud's phenomenon, and venous disorders.

COPD

Chronic obstructive pulmonary (or airways) disease (COPD) is a condition defined physiologically as airflow obstruction that generally results from a mixture of emphysema and peripheral airway obstruction due to chronic bronchitis (Senior & Shapiro, *Pulmonary Diseases and Disorders*, 3d ed., New York, McGraw-Hill, 1998, pp. 659-681, 1998; Barnes, *Chest 117*, 10S-14S, 2000). Emphysema is characterized by destruction of alveolar walls leading to abnormal enlargement of the air spaces of the lung. Chronic bronchitis is defined clinically as the presence of chronic productive cough for three months in each of two successive years. In COPD, airflow obstruction is usually progressive and is only partially reversible. By far the most important risk factor for development of COPD is cigarette smoking, although the disease does occur in non-smokers.

Chronic inflammation of the airways is a key pathological feature of COPD (Senior & Shapiro, 1998). The inflammatory cell population comprises increased numbers of macrophages, neutrophils, and $CD8^+$ lymphocytes. Inhaled irritants, such as cigarette smoke, activate macrophages which are resident in the respiratory tract, as well as epithelial cells leading to release of chemokines (*e.g.*, interleukin-8) and other chemotactic factors. These chemotactic factors act to increase the neutrophil/monocyte trafficking from the blood into the lung tissue and airways. Neutrophils and monocytes recruited into the airways can release a variety of potentially damaging mediators such as proteolytic enzymes and reactive oxygen species. Matrix degradation and emphysema, along with airway wall thickening, surfactant dysfunction, and mucus hypersecretion, all are potential sequelae of this inflammatory response that lead to impaired airflow and gas exchange.

GPCRs and COPD

Several GPCRs have been implicated in the pathology of COPD. For example, the chemokine IL-8 acts through CXCR1 and CXCR2, and antagonists for these receptors are under investigation as therapeutics for COPD. Members of the P2Y family of metabotropic receptors may play key roles in normal pulmonary function. In particular, the $P2Y_2$ receptor is believed to be involved in the regulation of mucociliary clearance mechanisms in the lung, and agonists of this receptor may stimulate airway mucus clearance in patients with chronic bronchitis (Yerxa Johnson, *Drugs of the Future* 24, 759-769, 1999). GPCRs, therefore, are therapeutic targets for COPD, and the identification of additional members of existing GPCR families or of novel GPCRs would yield further attractive targets.

Cancer

Cancer is a disease fundamentally caused by oncogenic cellular transformation. There are several hallmarks of transformed cells that distinguish them from their normal counterparts and underlie the pathophysiology of cancer. These include un-controlled cellular proliferation, unresponsiveness to normal death-inducing signals (immortalization), increased cellular motility and invasiveness, increased ability to recruit blood supply through induction of new blood vessel formation (angiogenesis), genetic instability, and dysregulated gene expression. Various combinations of these aberrant physiologies, along with the acquisition of drug-resistance frequently lead to an intractable disease state in which organ failure and patient death ultimately ensue.

Most standard cancer therapies target cellular proliferation and rely on the differential proliferative capacities between transformed and normal cells for their efficacy. This approach is hindered by the facts that several important normal cell types are also highly proliferative and that cancer cells frequently become resistant to

these agents. Thus, the therapeutic indices for traditional anti-cancer therapies rarely exceed 2.0.

The advent of genomics-driven molecular target identification has opened up the possibility of identifying new cancer-specific targets for therapeutic intervention that will provide safer, more effective treatments for cancer patients. Thus, newly discovered tumor-associated genes and their products can be tested for their role(s) in disease and used as tools to discover and develop innovative therapies. Genes playing important roles in any of the physiological processes outlined above can be characterized as cancer targets.

Genes or gene fragments identified through genomics can readily be expressed in one or more heterologous expression systems to produce functional recombinant proteins. These proteins are characterized *in vitro* for their biochemical properties and then used as tools in high-throughput molecular screening programs to identify chemical modulators of their biochemical activities. Agonists and/or antagonists of target protein activity can be identified in this manner and subsequently tested in cellular and *in vivo* disease models for anti-cancer activity. Optimization of lead compounds with iterative testing in biological models and detailed pharmacokinetic and toxicological analyses form the basis for drug development and subsequent testing in humans.

Allergy and Asthma

Allergy is a complex process in which environmental antigens induce clinically adverse reactions. The inducing antigens, called allergens, typically elicit a specific IgE response and, although in most cases the allergens themselves have little or no intrinsic toxicity, they induce pathology when the IgE response in turn elicits an IgE-dependent or T cell-dependent hypersensitivity reaction. Hypersensitivity reactions can be local or systemic and typically occur within minutes of allergen ex-

posure in individuals who have previously been sensitized to an allergen. The hypersensitivity reaction of allergy develops when the allergen is recognized by IgE antibodies bound to specific receptors on the surface of effector cells, such as mast cells, basophils, or eosinophils, which causes the activation of the effector cells and the release of mediators that produce the acute signs and symptoms of the reactions. Allergic diseases include asthma, allergic rhinitis (hay fever), atopic dermatitis, and anaphylaxis.

Asthma is though to arise as a result of interactions between multiple genetic and environmental factors and is characterized by three major features: 1) intermittent and reversible airway obstruction caused by bronchoconstriction, increased mucus production, and thickening of the walls of the airways that leads to a narrowing of the airways, 2) airway hyperresponsiveness caused by a decreased control of airway caliber, and 3) airway inflammation. Certain cells are critical to the inflammatory reaction of asthma and they include T cells and antigen presenting cells, B cells that produce IgE, and mast cells, basophils, eosinophils, and other cells that bind IgE. These effector cells accumulate at the site of allergic reaction in the airways and release toxic products that contribute to the acute pathology and eventually to the tissue destruction related to the disorder. Other resident cells, such as smooth muscle cells, lung epithelial cells, mucus-producing cells, and nerve cells may also be abnormal in individuals with asthma and may contribute to the pathology. While the airway obstruction of asthma, presenting clinically as an intermittent wheeze and shortness of breath, is generally the most pressing symptom of the disease requiring immediate treatment, the inflammation and tissue destruction associated with the disease can lead to irreversible changes that eventually make asthma a chronic disabling disorder requiring long-term management.

Despite recent important advances in our understanding of the pathophysiology of asthma, the disease appears to be increasing in prevalence and severity (Gergen and Weiss, *Am. Rev. Respir. Dis. 146*, 823-24, 1992). It is estimated that 30-40% of the

population suffer with atopic allergy, and 15% of children and 5% of adults in the population suffer from asthma (Gergen and Weiss, 1992). Thus, an enormous burden is placed on our health care resources. However, both diagnosis and treatment of asthma are difficult. The severity of lung tissue inflammation is not easy to measure and the symptoms of the disease are often indistinguishable from those of respiratory infections, chronic respiratory inflammatory disorders, allergic rhinitis, or other respiratory disorders. Often, the inciting allergen cannot be determined, making removal of the causative environmental agent difficult. Current pharmacological treatments suffer their own set of disadvantages. Commonly used therapeutic agents, such as beta agonists, can act as symptom relievers to transiently improve pulmonary function, but do not affect the underlying inflammation. Agents that can reduce the underlying inflammation, such as anti-inflammatory steroids, can have major drawbacks that range from immunosuppression to bone loss (Goodman and Gilman's THE PHARMACOLOGIC BASIS OF THERAPEUTICS, Seventh Edition, MacMillan Publishing Company, NY, USA, 1985). In addition, many of the present therapies, such as inhaled corticosteroids, are short-lasting, inconvenient to use, and must be used often on a regular basis, in some cases for life, making failure of patients to comply with the treatment a major problem and thereby reducing their effectiveness as a treatment.

Because of the problems associated with conventional therapies, alternative treatment strategies have been evaluated. Glycophorin A (Chu and Sharom, *Cell. Immunol. 145*, 223-39, 1992), cyclosporin (Alexander *et al.*, *Lancet 339*, 324-28, 1992), and a nonapeptide fragment of IL-2 (Zav'yalov *et al.*, *Immunol. Lett. 31*, 285-88, 1992) all inhibit interleukin-2 dependent T lymphocyte proliferation; however, they are known to have many other effects. For example, cyclosporin is used as a immunosuppressant after organ transplantation. While these agents may represent alternatives to steroids in the treatment of asthmatics, they inhibit interleukin-2 dependent T lymphocyte proliferation and potentially critical immune functions associated with homeostasis. Other treatments that block the release or activity of mediators of

bronchochonstriction, such as cromones or anti-leukotrienes, have recently been introduced for the treatment of mild asthma, but they are expensive and not effective in all patients and it is unclear whether they have any effect on the chronic changes associated with asthmatic inflammation. What is needed in the art is the identification of a treatment that can act in pathways critical to the development of asthma that both blocks the episodic attacks of the disorder and preferentially dampens the hyperactive allergic immune response without immunocompromising the patient.

GPCRs and asthma

G protein-coupled receptors (GPCRs) are membrane proteins characterized by seven transmembrane spanning domains that mediate the actions of many extracellular signals. GPCRs interact with heterotrimeric guanine nucleotide binding regulatory proteins (G proteins) that modulate a variety of second messenger systems or ionic conductances to effect physiological responses. Many of the mediators involved in airway smooth muscle contraction and in the chemoattraction of inflammatory cells exert their effects through GPCR binding. Among the mediators of smooth muscle contraction are leukotrienes, platelet-activating factor, endothelin-1, adenosine, and thromboxane A2. Receptor antagonists that block the activation of GPCRs by some of these mediators have been successfully used as treatments for asthma. Among the chemoattractants of inflammatory cells are the chemokines, such as eotaxin, MCP-4, RANTES, and IL-8. Chemokine receptor antagonists similarly are being developed as treatments for asthma. Sarau *et al.*, *Mol. Pharmacol. 56*, 657-63, 1999; Kitaura *et al.*, *J. Biol. Chem. 271*, 7725-30, 1996; Ligget *et al.*, *Am. J. Respir. Crit. Care Med. 152*, 394-402, 1995; Panettieri *et al.*, *J. Immunol. 154*, 2358-65, 1995; Noveral *et al.*, *Am. J. Physiol. 263*, L317-24, 1992; Honda *et al.*, *Nature 349*, 342-46, 1991.

Activation of some GPCRs may conversely have beneficial effects in asthma. For example, receptor agonists that activate the β1- and β2-adrenergic GPCRs are used therapeutically to relax contracted airway smooth muscle in the treatment of asthma

attacks. Thus, regulation of GPCRs in either a positive or negative manner may play an important role in the treatment of asthma.

Hematological disorders

Guanin-nucleotide-binding (G-) protein coupled receptors (GPCRs) are involved in various hematopoietic processes, e.g. proliferation, differentiation, survival, migration and homing of precursor cells to hematopoietic and lymphoid tissues. Dysfunction of GPCRs may lead to inappropriate production of blood cells resulting in diseases like anemia, leukopenia, thrombocytopenia or different forms of leukemia.

GPCRs also play a role in diverse functions of circulating white blood cells, e.g. activation of immune response in lymphocytes, cytokine production by monocytes and chemotaxis of granulocytes. Dysregulated GPCR function may contribute to compromised immune function, allergy and other pathologic conditions of the host defense system.

In circulating platelets GPCRs mediate activation resulting in platelet aggregation and secretion of mediators eventually leading to hemostasis. Modulation of GPCR function in platelets by pharmacologic or molecular genetic methods has demonstrated key roles of GPCRs in thrombotic diseases and in bleeding disorders thus proving that GPCRs represent appropriate therapeutic drug targets.

GPCRs are activated by binding of various classes of ligands ranging from small molecules like serotonin to high molecular peptides like chemokines. Some GPCRs are activated by proteolytic cleavage, e.g. by thrombin. Upon ligand binding, signals from GPCRs are mediated via heterotrimeric G-proteins with the class of the α-subunit determining the further pathway signal transduction.

It is conceivable that genes coding for „non-standard" GPCRs with unidentified ligands or unknown intracellular signal transduction pathways (e.g. novel G-proteins) or that GPCRs from classes as yet not associated with the hematopoietic and hemostatic systems will be identified. Therefore it is reasonable to assume that GPCRs specifically expressed in hematopoietic precursor or circulating blood cells represent good targets for therapeutic interventions in dysfunctions of hematopoiesis and hemostasis. See Yang M., Srikaiatkhachorn A, Antony M., Chong B.H.; Blood Coagul. Fibrinolysis 1996, 127-33; Arai H., Tsou C.L., Charo I.F.; Proc. Natl. Acad. Sci. USA 94, 14495-14499, 1997; Aragay A.M., Quick M.W.; J. Biol. Chem. 274, 4807-4815, 1999; Davignon I., Catalina M.D., Smith D., Montgomery J., Croy J., Siegelman M., Wilkie T.M.; Mol. Cell. Biol. 20, 797-804, 2000; Wiesmann A., Spangrude G.J.; Exp. Hematol. 27, 946-955, 1999; Van Brocklyn J.R., Graler M.H., Bernhardt G., Hobson J.P., Lipp M., Spiegel S.; Blood 95, 2624-2629, 2000; Brass L.F.; J. Clin. Invest. 104, 1663-1665, 1999; Coughlin S.R.; Proc. Natl. Acad. Sci. USA 96, 11023-11027, 1999.

Urological disorders.

Urinary Incontinence

Urinary incontinence (UI) is the involuntary loss of urine. Urge urinary incontinence (UUI) is one of the most common types of UI together with stress urinary incontinence (SUI), which is usually caused by a defect in the urethral closure mechanism. UUI is often associated with neurological disorders or diseases causing neuronal damage, such as dementia, Parkinson's disease, multiple sclerosis, stroke, and diabetes, although it also occurs in individuals with no such disorders. One of the usual causes of UUI is overactive bladder (OAB), which is a medical condition referring to the symptoms of frequency and urgency derived from abnormal contractions and instability of the detrusor muscle.

There are several medications for urinary incontinence on the market today, mainly to help treating UUI. Therapy for OAB is focused on drugs that affect peripheral neural control mechanisms or those that act directly on bladder detrusor smooth muscle contraction, with a major emphasis on development of anticholinergic agents. These agents can inhibit the parasympathetic nerves, which control bladder voiding, or can exert a direct spasmolytic effect on the detrusor muscle of the bladder. This results in a decrease in intravesicular pressure, an increase in capacity, and a reduction in the frequency of bladder contraction. Orally active anticholinergic drugs, such as propantheline (ProBanthine), tolterodine tartrate (Detrol), and oxybutynin (Ditropan), are the most commonly prescribed drugs. However, their most serious drawbacks are unacceptable side effects, such as dry mouth, abnormal visions, constipation, and central nervous system disturbances. These side effects lead to poor compliance. Dry mouth symptoms alone are responsible for a 70% non-compliance rate with oxybutynin. The inadequacies of present therapies highlight the need for novel, efficacious, safe, orally available drugs that have fewer side effects.

Serotonin receptors

Correctly coordinated detrusor relaxation and contraction of the urethral sphincter are required for the normal bladder filling, while voiding requires the converse. This harmonized coordination is achieved by the integration of excitatory, inhibitory, and sensory nerve activities in micturition centers located in the spinal cord, pons, and forebrain. Several neurotransmitters, such as 5-hydroxytryptamine (5-HT), □-aminobutyric acid, glycine, dopamine, acetylcholine, and enkephalins, have been identified in the micturition reflex pathways at both spinal and supraspinal sites (de Groat *et al.*, Nervous Control of the Urogenital System, 227-290, 1993).

It has been recognized that 5-HT has an effect on micturition through both central and peripheral mechanisms (Espey & Downie, Eur J Pharmacol 287: 173-177, 1995).

Most of the well-characterized 5-HT receptors are G-protein coupled receptors (Raymond et al., Naunyn Schmiedebergs Arch Pharmacol 346: 127-137, 1992). The 5-HT1 family consists of five receptors (5-HT1A, 1B, 1D, 1E and 1F) that share the property of inhibiting the enzyme adenylate cyclase when stimulated by serotonin. With the exception of the 5-HT1E receptor, which appears to be limited to the CNS, these receptors are found in both the CNS and the periphery.

The physiological role of 5-HT1A in the control of micturition was elucidated using 5-HT1A receptor selective antagonist WAY100635, which showed a marked block of isovolumetric bladder contraction in anesthetized rats and an increase of bladder capacity without consistently impairing bladder contractility in conscious rats (Lecci et al., J Pharmacol Exp Therap 262: 181-189, 1992. Blockade of spinal 5-HT1A receptors by intrathecal administration of WAY100635 inhibited the micturition reflex induced by bladder distension as well as bladder contractions elicited by electrical stimulation of the pontine micturition center, whereas it did not show any effect on the ascending pathway. The effective intrathecal administration of WAY100635 was restricted to the L6-S1 spinal cord level Hedge & Eglen, FASEB J 10: 1398-1407, 1996. It was also shown that intrathecal administration of 5-HT1A receptor selective agonist 8-OH-DPAT facilitated the micturition reflex in normal rats and that intravenous administration of 8-OH-DPAT increased the amplitude of reflex-bladder contractions induced by bladder distension in chronically spinalized rats (Khan et al., World J Urol 17:255-260, 1999). Taken together with these results 5-HT1A receptors at the lumbosacral spinal cord level have an important role in tonic control of the micturition reflex pathway. 5-HT1A antagonists may provide therapeutic benefit to treat overactive bladder and urge urinary incontinence.

The 5-HT2 family consists of three receptors (5-HT2A, 2B and 2C) that act through increasing intracellular phosphoinositide metabolism. The 5-HT4 receptor is present in both the CNS and on various tissues of the periphery, where it is positively coupled to adenylate cyclase. There appears to be a negative coupling of the 5-HT5

receptor to adenylate cyclase. The 5-HT6 and 5-HT7 receptors are both positively coupled to adenylate cyclase. The 5-HT6 receptor is found only in the CNS, while 5-HT7 receptors are located in both central and peripheral tissues. The only serotonin receptor that is a member of the ligand-gated ion channel superfamily is the 5-HT3 receptor. When stimulated by serotonin, this receptor conducts a depolarizing cation current into cells.

The activation of 5-HT3 or 5-HT4 receptors facilitates acetylcholine release (Testa et al., J Pharmacol Exp Ther 290: 1258-1269, 1999. 5-HT receptors are upregulated in the bladder following obstruction (Kakizaki et al., Am J Physiol Regulatory Integrative Comp Physiol 280: R1407-1413, 2001. Thus, 5-HT3 or 5-HT4 antagonists may be beneficial in the treatment of the overactive bladder.

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a modulating agent, an antisense nucleic acid molecule, a specific antibody, ribozyme, or a 5-HT-like GPCR polypeptide binding molecule) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

A reagent which affects 5-HT-like GPCR activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce 5-HT-like GPCR activity. The reagent preferably binds to an expression product of a human 5-HT-like GPCR gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells which

have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain, lymph nodes, and skin.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a tumor cell, such as a tumor cell ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μg to about 5 μg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al. Trends in Biotechnol. 11*, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem. 263*, 621-24 (1988); Wu *et al.*, *J. Biol. Chem. 269*, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem. 266*, 338-42 (1991).

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases 5-HT-like GPCR activity relative to the 5-HT-like GPCR activity which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}.

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 μg to about 50 μg/kg, about 50 μg to about 5 mg/kg, about 100 μg to about 500 μg /kg of patient body weight, and about 200 to about 250 μg /kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20 μg to about 100 μg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of a 5-HT-like GPCR gene or the activity of a 5-HT-like GPCR polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a 5-HT-like GPCR gene or the activity of a 5-HT-like GPCR polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to 5-HT-like GPCR-specific mRNA, quantitative RT-PCR, immunologic detection of a 5-HT-like GPCR polypeptide, or measurement of 5-HT-like GPCR activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Diagnostic Methods

GPCRs also can be used in diagnostic assays for detecting diseases and abnormalities or susceptibility to diseases and abnormalities related to the presence of mutations in the nucleic acid sequences which encode a GPCR. Such diseases, by way of example, are related to cell transformation, such as tumors and cancers, and various cardiovascular disorders, including hypertension and hypotension, as well as diseases arising from abnormal blood flow, abnormal angiotensin-induced aldosterone secretion, and other abnormal control of fluid and electrolyte homeostasis.

Differences can be determined between the cDNA or genomic sequence encoding a GPCR in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized, for example, by high resolution gel electrophoresis. DNA fragments of different sequences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (*see, e.g.*, Myers *et al.*, *Science 230*, 1242, 1985). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (*e.g.*, Cotton *et al.*, *Proc. Natl. Acad. Sci. USA 85*, 4397-4401, 1985). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes and Southern blotting of genomic DNA. In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

Altered levels of a GPCR also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Detection of 5-HT-GPCR activity

The polynucleotide of SEQ ID NO: 1 is inserted into the expression vector pCEV4 and the expression vector pCEV4-5-HT-GPCR polypeptide obtained is transfected into human embryonic kidney 293 cells. The cells are scraped from a culture flask into 5 ml of Tris HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. Cell lysates are centrifuged at 1000 rpm for 5 minutes at 4 °C. The supernatant is centrifuged at 30,000 x g for 20 minutes at 4 °C. The pellet is suspended in binding buffer containing 50 mM Tris HCl, 5 mM $MgSO_4$, 1 mM EDTA, 100 mM NaCl, pH 7.5, supplemented with 0.1 % BSA, 2 µg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 µg/ml phosphoramidon. Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10 % of an added radioligand, i.e. ^{125}I-labeled serotonin, are added to 96-well polypropylene microtiter plates containing ligand, non-labeled peptides, and binding buffer to a final volume of 250 µl.

In equilibrium saturation binding assays, membrane preparations are incubated in the presence of increasing concentrations (0.1 nM to 4 nM) of ^{125}I ligand.

Binding reaction mixtures are incubated for one hour at 30 °C. The reaction is stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Radioactivity is measured by scintillation counting, and data are analyzed by a computerized non-linear regression program. Non-specific binding is defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 100 nM of unlabeled peptide. Protein concentration is measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a standard. The 5-HT-GPCR activity of the polypeptide comprising the amino acid sequence of SEQ ID NO: 2 is demonstrated.

EXAMPLE 2

Radioligand binding assays

Human embryonic kidney 293 cells transfected with a polynucleotide which expresses human 5-HT-like GPCR are scraped from a culture flask into 5 ml of Tris HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. Cell lysates are centrifuged at 1000 rpm for 5 minutes at 4 °C. The supernatant is centrifuged at 30,000 x g for 20 minutes at 4 °C. The pellet is suspended in binding buffer containing 50 mM Tris HCl, 5 mM $MgSO_4$, 1 mM EDTA, 100 mM NaCl, pH 7.5, supplemented with 0.1 % BSA, 2 µg /ml aprotinin, 0.5 mg/ml leupeptin, and 10 µg /ml phosphoramidon. Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10 % of the added radioligand, are added to 96-well polypropylene microtiter plates containing ^{125}I-labeled ligand or test compound, non-labeled peptides, and binding buffer to a final volume of 250 µl.

In equilibrium saturation binding assays, membrane preparations are incubated in the presence of increasing concentrations (0.1 nM to 4 nM) of ^{125}I-labeled ligand or test compound (specific activity 2200 Ci/mmol). The binding affinities of different test compounds are determined in equilibrium competition binding assays, using 0.1 nM ^{125}I-peptide in the presence of twelve different concentrations of each test compound.

Binding reaction mixtures are incubated for one hour at 30 °C. The reaction is stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Radioactivity is measured by scintillation counting, and data are analyzed by a computerized non-linear regression program.

Non-specific binding is defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 100 nM of unlabeled peptide. Protein concentration is measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a standard. A test compound which increases the

radioactivity of membrane protein by at least 15% relative to radioactivity of membrane protein which was not incubated with a test compound is identified as a compound which binds to a human 5-HT-like GPCR polypeptide.

EXAMPLE 3

Effect of a test compound on human 5-HT-like GPCR -mediated cyclic AMP formation

Receptor-mediated inhibition of cAMP formation can be assayed in host cells which express human 5-HT-like GPCR. Cells are plated in 96-well plates and incubated in Dulbecco's phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 5 mM theophylline, 2 μg /ml aprotinin, 0.5 mg/ml leupeptin, and 10 μg /ml phosphoramidon for 20 minutes at 37 °C in 5% CO2. A test compound is added and incubated for an additional 10 minutes at 37 °C. The medium is aspirated, and the reaction is stopped by the addition of 100 mM HCl. The plates are stored at 4 °C for 15 minutes. cAMP content in the stopping solution is measured by radioimmunoassay.

Radioactivity is quantified using a gamma counter equipped with data reduction software. A test compound which decreases radioactivity of the contents of a well relative to radioactivity of the contents of a well in the absence of the test compound is identified as a potential inhibitor of cAMP formation. A test compound which increases radioactivity of the contents of a well relative to radioactivity of the contents of a well in the absence of the test compound is identified as a potential enhancer of cAMP formation.

EXAMPLE 4

Effect of a test compound on the mobilization of intracellular calcium

Intracellular free calcium concentration can be measured by microspectrofluorometry using the fluorescent indicator dye Fura-2/AM (Bush *et al., J. Neurochem. 57,* 562-

74, 1991). Stably transfected cells are seeded onto a 35 mm culture dish containing a glass coverslip insert. Cells are washed with HBS , incubated with a test compound, and loaded with 100 µl of Fura-2/AM (10 µM) for 20-40 minutes. After washing with HBS to remove the Fura-2/AM solution, cells are equilibrated in HBS for 10-20 minutes. Cells are then visualized under the 40X objective of a Leitz Fluovert FS microscope.

Fluorescence emission is determined at 510 nM, with excitation wavelengths alternating between 340 nM and 380 nM. Raw fluorescence data are converted to calcium concentrations using standard calcium concentration curves and software analysis techniques. A test compound which increases the fluorescence by at least 15% relative to fluorescence in the absence of a test compound is identified as a compound which mobilizes intracellular calcium.

EXAMPLE 5

Effect of a test compound on phosphoinositide metabolism

Cells which stably express human 5-HT-like GPCR cDNA are plated in 96-well plates and grown to confluence. The day before the assay, the growth medium is changed to 100 µl of medium containing 1% serum and 0.5 µCi ^{3}H-myinositol. The plates are incubated overnight in a CO_2 incubator (5% CO_2 at 37 °C). Immediately before the assay, the medium is removed and replaced by 200 µl of PBS containing 10 mM LiCl, and the cells are equilibrated with the new medium for 20 minutes. During this interval, cells also are equilibrated with antagonist, added as a 10 µl aliquot of a 20-fold concentrated solution in PBS.

The ^{3}H-inositol phosphate accumulation from inositol phospholipid metabolism is started by adding 10 µl of a solution containing a test compound. To the first well 10 µl are added to measure basal accumulation. Eleven different concentrations of test compound are assayed in the following 11 wells of each plate row. All assays are

performed in duplicate by repeating the same additions in two consecutive plate rows.

The plates are incubated in a CO_2 incubator for one hour. The reaction is terminated by adding 15 µl of 50% v/v trichloroacetic acid (TCA), followed by a 40 minute incubation at 4 °C. After neutralizing TCA with 40 µl of 1 M Tris, the content of the wells is transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates are prepared by adding 200 µl of Dowex AG1-X8 suspension (50% v/v, water:resin) to each well. The filter plates are placed on a vacuum manifold to wash or elute the resin bed. Each well is washed 2 times with 200 µl of water, followed by 2 x 200 µl of 5 mM sodium tetraborate/60 mM ammonium formate.

The ^{3}H-IPs are eluted into empty 96-well plates with 200 µl of 1.2 M ammonium formate/0.1 formic acid. The content of the wells is added to 3 ml of scintillation cocktail, and radioactivity is determined by liquid scintillation counting.

EXAMPLE 6

Receptor Binding Methods

Standard Binding Assays. Binding assays are carried out in a binding buffer containing 50 mM HEPES, pH 7.4, 0.5% BSA, and 5 mM $MgCl_2$. The standard assay for radioligand binding to membrane fragments comprising 5-HT-like GPCR polypeptides is carried out as follows in 96 well microtiter plates (*e.g.*, Dynatech Immulon II Removawell plates). Radioligand is diluted in binding buffer+ PMSF/Baci to the desired cpm per 50 µl, then 50 µl aliquots are added to the wells. For non-specific binding samples, 5 µl of 40 µM cold ligand also is added per well. Binding is initiated by adding 150 µl per well of membrane diluted to the desired concentration (10-30 µg membrane protein/well) in binding buffer+ PMSF/Baci. Plates are then covered with Linbro mylar plate sealers (Flow Labs) and placed on a

Dynatech Microshaker II. Binding is allowed to proceed at room temperature for 1-2 hours and is stopped by centrifuging the plate for 15 minutes at 2,000 x g. The supernatants are decanted, and the membrane pellets are washed once by addition of 200 µl of ice cold binding buffer, brief shaking, and recentrifugation. The individual wells are placed in 12 x 75 mm tubes and counted in an LKB Gammamaster counter (78% efficiency). Specific binding by this method is identical to that measured when free ligand is removed by rapid (3-5 seconds) filtration and washing on polyethylene-imine-coated glass fiber filters.

Three variations of the standard binding assay are also used.

1. Competitive radioligand binding assays with a concentration range of cold ligand vs. ^{125} I-labeled ligand are carried out as described above with one modification. All dilutions of ligands being assayed are made in 40X PMSF/Baci to a concentration 40X the final concentration in the assay. Samples of peptide (5 µl each) are then added per microtiter well. Membranes and radioligand are diluted in binding buffer without protease inhibitors. Radioligand is added and mixed with cold ligand, and then binding is initiated by addition of membranes.

2. Chemical cross-linking of radioligand with receptor is done after a binding step identical to the standard assay. However, the wash step is done with binding buffer minus BSA to reduce the possibility of non-specific cross-linking of radioligand with BSA. The cross-linking step is carried out as described below.

3. Larger scale binding assays to obtain membrane pellets for studies on solubilization of receptor:ligand complex and for receptor purification are also carried out. These are identical to the standard assays except that (a) binding is carried out in polypropylene tubes in volumes from 1-250 ml, (b) concentration of membrane protein is always 0.5 mg/ml, and (c) for receptor purification, BSA concentration in the binding buffer is reduced to 0.25%, and the wash step is done

with binding buffer without BSA, which reduces BSA contamination of the purified receptor.

EXAMPLE 7

Chemical Cross-Linking of Radioligand to Receptor

After a radioligand binding step as described above, membrane pellets are re-suspended in 200 µl per microtiter plate well of ice-cold binding buffer without BSA. Then 5 µl per well of 4 mM N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOS, Pierce) in DMSO is added and mixed. The samples are held on ice and UV-irradiated for 10 minutes with a Mineralight R-52G lamp (UVP Inc., San Gabriel, Calif.) at a distance of 5-10 cm. Then the samples are transferred to Eppendorf microfuge tubes, the membranes pelleted by centrifugation, supernatants removed, and membranes solubilized in Laemmli SDS sample buffer for polyacrylamide gel electrophoresis (PAGE). PAGE is carried out as described below. Radiolabeled proteins are visualized by autoradiography of the dried gels with Kodak XAR film and Dupont image intensifier screens.

EXAMPLE 8

Membrane Solubilization

Membrane solubilization is carried out in buffer containing 25 mM Tris , pH 8, 10% glycerol (w/v) and 0.2 mM $CaCl_2$ (solubilization buffer). The highly soluble detergents including Triton X-100, deoxycholate, deoxycholate:lysolecithin, CHAPS, and zwittergent are made up in solubilization buffer at 10% concentrations and stored as frozen aliquots. Lysolecithin is made up fresh because of insolubility upon freeze-thawing and digitonin is made fresh at lower concentrations due to its more limited solubility.

To solubilize membranes, washed pellets after the binding step are resuspended free of visible particles by pipetting and vortexing in solubilization buffer at 100,000 x g for 30 minutes. The supernatants are removed and held on ice and the pellets are discarded.

EXAMPLE 9

Assay of Solubilized Receptors

After binding of ^{125}I ligands and solubilization of the membranes with detergent, the intact R:L complex can be assayed by four different methods. All are carried out on ice or in a cold room at 4-10 °C.).

1. Column chromatography (Knuhtsen *et al.*, *Biochem. J. 254*, 641-647, 1988). Sephadex G-50 columns (8 x 250 mm) are equilibrated with solubilization buffer containing detergent at the concentration used to solubilize membranes and 1 mg/ml bovine serum albumin. Samples of solubilized membranes (0.2-0.5 ml) are applied to the columns and eluted at a flow rate of about 0.7 ml/minute. Samples (0.18 ml) are collected. Radioactivity is determined in a gamma counter. Void volumes of the columns are determined by the elution volume of blue dextran. Radioactivity eluting in the void volume is considered bound to protein. Radioactivity eluting later, at the same volume as free ^{125}I ligands, is considered non-bound.

2. Polyethyleneglycol precipitation (Cuatrecasas, *Proc. Natl. Acad. Sci. USA 69*, 318-322, 1972). For a 100 µl sample of solubilized membranes in a 12 x 75 mm polypropylene tube, 0.5 ml of 1% (w/v) bovine gamma globulin (Sigma) in 0.1 M sodium phosphate buffer is added, followed by 0.5 ml of 25% (w/v) polyethylene-glycol (Sigma) and mixing. The mixture is held on ice for 15 minutes. Then 3 ml of 0.1 M sodium phosphate, pH 7.4, is added per sample. The samples are rapidly (1-3 seconds) filtered over Whatman GF/B glass fiber filters and washed with 4 ml of the

phosphate buffer. PEG-precipitated receptor : ^{125} I-ligand complex is determined by gamma counting of the filters.

3. GFB/PEI filter binding (Bruns *et al.*, *Analytical Biochem. 132*, 74-81, 1983). Whatman GF/B glass fiber filters are soaked in 0.3% polyethyleneimine (PEI, Sigma) for 3 hours. Samples of solubilized membranes (25-100 µl) are replaced in 12 x 75 mm polypropylene tubes. Then 4 ml of solubilization buffer without detergent is added per sample and the samples are immediately filtered through the GFB/PEI filters (1-3 seconds) and washed with 4 ml of solubilization buffer. CPM of receptor : ^{125} I-ligand complex adsorbed to filters are determined by gamma counting.

4. Charcoal/Dextran (Paul and Said, *Peptides 7[Suppl. 1]*,147-149, 1986). Dextran T70 (0.5 g, Pharmacia) is dissolved in 1 liter of water, then 5 g of activated charcoal (Norit A, alkaline; Fisher Scientific) is added. The suspension is stirred for 10 minutes at room temperature and then stored at 4 °C. until use. To measure R:L complex, 4 parts by volume of charcoal/dextran suspension are added to 1 part by volume of solubilized membrane. The samples are mixed and held on ice for 2 minutes and then centrifuged for 2 minutes at 11,000 x g in a Beckman microfuge. Free radioligand is adsorbed charcoal/dextran and is discarded with the pellet. Receptor : ^{125} I-ligand complexes remain in the supernatant and are determined by gamma counting.

EXAMPLE 10

Receptor Purification

Binding of biotinyl-receptor to GH_4 C1 membranes is carried out as described above. Incubations are for 1 hour at room temperature. In the standard purification protocol, the binding incubations contain 10 nM Bio-S29. ^{125}I ligand is added as a tracer at

levels of 5,000-100,000 cpm per mg of membrane protein. Control incubations contain 10 μM cold ligand to saturate the receptor with non-biotinylated ligand.

Solubilization of receptor:ligand complex also is carried out as described above, with 0.15% deoxycholate:lysolecithin in solubilization buffer containing 0.2 mM $MgCl_2$, to obtain 100,000 x g supernatants containing solubilized R:L complex.

Immobilized streptavidin (streptavidin cross-linked to 6% beaded agarose, Pierce Chemical Co.; "SA-agarose") is washed in solubilization buffer and added to the solubilized membranes as 1/30 of the final volume. This mixture is incubated with constant stirring by end-over-end rotation for 4-5 hours at 4-10 °C. Then the mixture is applied to a column and the non-bound material is washed through. Binding of radioligand to SA-agarose is determined by comparing cpm in the 100,000 x g supernatant with that in the column effluent after adsorption to SA-agarose. Finally, the column is washed with 12-15 column volumes of solubilization buffer+0.15% deoxycholate:lysolecithin +1/500 (vol/vol) 100 x 4pase.

The streptavidin column is eluted with solubilization buffer+0.1 mM EDTA+0.1 mM EGTA+0.1 mM GTP-gamma-S (Sigma)+0.15% (wt/vol) deoxycholate:lysolecithin +1/1000 (vol/vol) 100.times.4pase. First, one column volume of elution buffer is passed through the column and flow is stopped for 20-30 minutes. Then 3-4 more column volumes of elution buffer are passed through. All the eluates are pooled.

Eluates from the streptavidin column are incubated overnight (12-15 hours) with immobilized wheat germ agglutinin (WGA agarose, Vector Labs) to adsorb the receptor via interaction of covalently bound carbohydrate with the WGA lectin. The ratio (vol/vol) of WGA-agarose to streptavidin column eluate is generally 1:400. A range from 1:1000 to 1:200 also can be used. After the binding step, the resin is pelleted by centrifugation, the supernatant is removed and saved, and the resin is washed 3 times (about 2 minutes each) in buffer containing 50 mM HEPES, pH 8, 5

mM $MgCl_2$, and 0.15% deoxycholate:lysolecithin. To elute the WGA-bound receptor, the resin is extracted three times by repeated mixing (vortex mixer on low speed) over a 15-30 minute period on ice, with 3 resin columns each time, of 10 mM N-N'-N"-triacetylchitotriose in the same HEPES buffer used to wash the resin. After each elution step, the resin is centrifuged down and the supernatant is carefully removed, free of WGA-agarose pellets. The three, pooled eluates contain the final, purified receptor. The material non-bound to WGA contain G protein subunits specifically eluted from the streptavidin column, as well as non-specific contaminants. All these fractions are stored frozen at -90 °C.

EXAMPLE 11

Identification of test compounds that bind to 5-HT-like GPCR polypeptides

Purified 5-HT-like GPCR polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. 5-HT-like GPCR polypeptides comprise an amino acid sequence shown in SEQ ID NO:2. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a 5-HT-like GPCR polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound which increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound is not incubated is identified as a compound which binds to a 5-HT-like GPCR polypeptide.

EXAMPLE 12

Identification of a test compound which decreases 5-HT-like GPCR gene expression

A test compound is administered to a culture of human gastric cells and incubated at 37 °C for 10 to 45 minutes. A culture of the same type of cells incubated for the same time without the test compound provides a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18*, 5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and hybridized with a ^{32}P-labeled 5-HT-like GPCR-specific probe at 65 ° C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NO:1. A test compound which decreases the 5-HT-like GPCR-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of 5-HT-like GPCR gene expression.

EXAMPLE 13

Expression of recombinant human 5-HT-like GPCR

The *Pichia pastoris* expression vector pPICZB (Invitrogen, San Diego, CA) is used to produce large quantities of recombinant human 5-HT-like GPCR polypeptides in yeast. The 5-HT-like GPCR-encoding DNA sequence is derived from SEQ ID NO:1. Before insertion into vector pPICZB, the DNA sequence is modified by well known methods in such a way that it contains at its 5'-end an initiation codon and at its 3'-end an enterokinase cleavage site, a His6 reporter tag and a termination codon. Moreover, at both termini recognition sequences for restriction endonucleases are added and after digestion of the multiple cloning site of pPICZ B with the corresponding restriction enzymes the modified DNA sequence is ligated into pPICZB. This expression vector is designed for inducible expression in *Pichia pastoris*, driven by a yeast promoter. The resulting pPICZ/md-His6 vector is used to transform the yeast.

The yeast is cultivated under usual conditions in 5 liter shake flasks and the recombinantly produced protein isolated from the culture by affinity chromatography (Ni-NTA-Resin) in the presence of 8 M urea. The bound polypeptide is eluted with buffer, pH 3.5, and neutralized. Separation of the polypeptide from the His6 reporter tag is accomplished by site-specific proteolysis using enterokinase (Invitrogen, San Diego, CA) according to manufacturer's instructions. Purified human 5-HT-like GPCR polypeptide is obtained.

EXAMPLE 14

Tissue-specific expression of 5-HT-like GPCR

The qualitative expression pattern of 5-HT-like GPCR in various tissues and cell lines was determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

To demonstrate that 5-HT-like GPCR is involved in CNS disorders, the following tissues are screened: fetal and adult brain, muscle, heart, lung, kidney, liver, thymus, testis, colon, placenta, trachea, pancreas, kidney, gastric mucosa, colon, liver, cerebellum, skin, cortex (Alzheimer's and normal), hypothalamus, cortex, amygdala, cerebellum, hippocampus, choroid, plexus, thalamus, and spinal cord.

To demonstrate that 5-HT-like GPCR is involved in the disease process of obesity, expression is determined in the following tissues: subcutaneous adipose tissue, mesenteric adipose tissue, adrenal gland, bone marrow, brain (cerebellum, spinal cord, cerebral cortex, caudate, medulla, substantia nigra, and putamen), colon, fetal brain, heart, kidney, liver, lung, mammary gland, pancreas, placenta, prostate, salivary gland, skeletal muscle small intestine, spleen, stomach, testes, thymus, thyroid trachea, and uterus. Neuroblastoma cell lines SK-Nr-Be (2), Hr, Sk-N-As, HTB-10, IMR-32, SNSY-5Y, T3, SK-N-D2, D283, DAOY, CHP-2, U87MG,

BE(2)C, T986, KANTS, MO59K, CHP234, C6 (rat), SK-N-F1, SK-PU-DW, PFSK-1, BE(2)M17, and MCIXC also are tested for 5-HT-like GPCR expression. As a final step, the expression of 5-HT-like GPCR in cells derived from normal individuals with the expression of cells derived from obese individuals is compared.

To demonstrate that 5-HT-like GPCR is involved in the disease process of COPD, the initial expression panel consists of RNA samples from respiratory tissues and inflammatory cells relevant to COPD: lung (adult and fetal), trachea, freshly isolated alveolar type II cells, cultured human bronchial epithelial cells, cultured small airway epithelial cells, cultured bronchial sooth muscle cells, cultured H441 cells (Clara-like), freshly isolated neutrophils and monocytes, and cultured monocytes (macrophage-like). Body map profiling also is carried out, using total RNA panels purchased from Clontech. The tissues are adrenal gland, bone marrow, brain, colon, heart, kidney, liver, lung, mammary gland, pancreas, prostate, salivary gland, skeletal muscle, small intestine, spleen, stomach, testis, thymus, trachea, thyroid, and uterus.

To demonstrate that 5-HT-like GPCR is involved in cancer, expression is determined in the following tissues: adrenal gland, bone marrow, brain, cerebellum, colon, fetal brain, fetal liver, heart, kidney, liver, lung, mammary gland, pancreas, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thymus, thyroid, trachea, uterus, and peripheral blood lymphocytes. Expression in the following cancer cell lines also is determined: DU-145 (prostate), NCI-H125 (lung), HT-29 (colon), COLO-205 (colon), A-549 (lung), NCI-H460 (lung), HT-116 (colon), DLD-1 (colon), MDA-MD-231 (breast), LS174T (colon), ZF-75 (breast), MDA-MN-435 (breast), HT-1080, MCF-7 (breast), and U87. Matched pairs of malignant and normal tissue from the same patient also are tested.

To demonstrate that 5-HT-like GPCR is involved in the disease process of diabetes, the following whole body panel is screened to show predominant or relatively high expression: subcutaneous and mesenteric adipose tissue, adrenal gland, bone marrow,

brain, colon, fetal brain, heart, hypothalamus, kidney, liver, lung, mammary gland, pancreas, placenta, prostate, salivary gland, skeletal muscle, small intestine, spleen, stomach, testis, thymus, thyroid, trachea, and uterus. Human islet cells and an islet cell library also are tested. As a final step, the expression of 5-HT-like GPCR in cells derived from normal individuals with the expression of cells derived from diabetic individuals is compared.

To demonstrate that 5-HT-like GPCR is involved in the disease process of asthma, the following whole body panel is screened to show predominant or relatively high expression in lung or immune tissues: brain, heart, kidney, liver, lung, trachea, bone marrow, colon, small intestine, spleen, stomach, thymus, mammary gland, skeletal muscle, prostate, testis, uterus, cerebellum, fetal brain, fetal liver, spinal cord, placenta, adrenal gland, pancreas, salivary gland, thyroid, peripheral blood leukocytes, lymph node, and tonsil. Once this is established, the following lung and immune system cells are screened to localize expression to particular cell subsets: lung microvascular endothelial cells, bronchial/tracheal epithelial cells, bronchial/tracheal smooth muscle cells, lung fibroblasts, T cells (T helper 1 subset, T helper 2 subset, NKT cell subset, and cytotoxic T lymphocytes), B cells, mononuclear cells (monocytes and macrophages), mast cells, eosinophils, neutrophils, and dendritic cells. As a final step, the expression of 5-HT-like GPCR in cells derived from normal individuals with the expression of cells derived from asthmatic individuals is compared.

Quantitative expression profiling. Quantitative expression profiling is performed by the form of quantitative PCR analysis called "kinetic analysis" firstly described in Higuchi *et al.*, *BioTechnology 10*, 413-17, 1992, and Higuchi *et al.*, *BioTechnology 11*, 1026-30, 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies.

If the amplification is performed in the presence of an internally quenched fluorescent oligonucleotide (TaqMan probe) complementary to the target sequence, the probe is cleaved by the 5'-3' endonuclease activity of Taq DNA polymerase and a fluorescent dye released in the medium (Holland *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 88*, 7276-80, 1991). Because the fluorescence emission will increase in direct proportion to the amount of the specific amplified product, the exponential growth phase of PCR product can be detected and used to determine the initial template concentration (Heid *et al.*, *Genome Res. 6*, 986-94, 1996, and Gibson *et al.*, *Genome Res. 6*, 995-1001, 1996).

The amplification of an endogenous control can be performed to standardize the amount of sample RNA added to a reaction. In this kind of experiment, the control of choice is the 18S ribosomal RNA. Because reporter dyes with differing emission spectra are available, the target and the endogenous control can be independently quantified in the same tube if probes labeled with different dyes are used.

All "real time PCR" measurements of fluorescence are made in the ABI Prism 7700.

RNA extraction and cDNA preparation. Total RNA from the tissues listed above are used for expression quantification. RNAs labeled "from autopsy" are extracted from autoptic tissues with the TRIzol reagent (Life Technologies, MD) according to the manufacturer's protocol.

Fifty µg of each RNA are treated with DNase I for 1 hour at 37°C in the following reaction mix: 0.2 U/µl RNase-free DNase I (Roche Diagnostics, Germany); 0.4 U/µl RNase inhibitor (PE Applied Biosystems, CA); 10 mM Tris-HCl pH 7.9; 10mM $MgCl_2$; 50 mM NaCl; and 1 mM DTT.

After incubation, RNA is extracted once with 1 volume of phenol:chloroform:isoamyl alcohol (24:24:1) and once with chloroform, and

precipitated with 1/10 volume of 3 M sodium acetate, pH5.2, and 2 volumes of ethanol.

Fifty μg of each RNA from the autoptic tissues are DNase treated with the DNA-free kit purchased from Ambion (Ambion, TX). After resuspension and spectrophotometric quantification, each sample is reverse transcribed with the TaqMan Reverse Transcription Reagents (PE Applied Biosystems, CA) according to the manufacturer's protocol. The final concentration of RNA in the reaction mix is 200ng/μL. Reverse transcription is carried out with 2.5μM of random hexamer primers.

TaqMan quantitative analysis. Specific primers and probe are designed according to the recommendations of PE Applied Biosystems; a FAM (6-carboxyfluorescein)- or TAMRA (6-carboxytetramethylrhodamine)-labeled probe is used Quantification experiments are performed on 10 ng of reverse transcribed RNA from each sample. Each determination is done in triplicate.

Total cDNA content is normalized with the simultaneous quantification (multiplex PCR) of the 18S ribosomal RNA using the Pre-Developed TaqMan Assay Reagents (PDAR) Control Kit (PE Applied Biosystems, CA).

The assay reaction mix is as follows: 1X final TaqMan Universal PCR Master Mix (from 2X stock) (PE Applied Biosystems, CA); 1X PDAR control – 18S RNA (from 20X stock); 300 nM forward primer; 900 nM reverse primer; 200 nM probe; 10 ng cDNA; and water to 25 ml.

Each of the following steps are carried out once: pre PCR, 2 minutes at 50°C, and 10 minutes at 95°C. The following steps are carried out 40 times: denaturation, 15 seconds at 95°C, annealing/extension, 1 minute at 60°C.

The experiment is performed on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR are processed as described in the ABI Prism 7700 user's manual in order to achieve better background subtraction as well as signal linearity with the starting target quantity.

EXAMPLE 15

In vivo testing of compounds/target validation

1. Pain:

Acute Pain

Acute pain is measured on a hot plate mainly in rats. Two variants of hot plate testing are used: In the classical variant animals are put on a hot surface (52 to 56 °C) and the latency time is measured until the animals show nocifensive behavior, such as stepping or foot licking. The other variant is an increasing temperature hot plate where the experimental animals are put on a surface of neutral temperature. Subsequently this surface is slowly but constantly heated until the animals begin to lick a hind paw. The temperature which is reached when hind paw licking begins is a measure for pain threshold.

Compounds are tested against a vehicle treated control group. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

Persistent Pain

Persistent pain is measured with the formalin or capsaicin test, mainly in rats. A solution of 1 to 5% formalin or 10 to 100 µg capsaicin is injected into one hind paw of the experimental animal. After formalin or capsaicin application the animals show

nocifensive reactions like flinching, licking and biting of the affected paw. The number of nocifensive reactions within a time frame of up to 90 minutes is a measure for intensity of pain.

Compounds are tested against a vehicle treated control group. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to formalin or capsaicin administration.

Neuropathic Pain

Neuropathic pain is induced by different variants of unilateral sciatic nerve injury mainly in rats. The operation is performed under anesthesia. The first variant of sciatic nerve injury is produced by placing loosely constrictive ligatures around the common sciatic nerve. The second variant is the tight ligation of about the half of the diameter of the common sciatic nerve. In the next variant, a group of models is used in which tight ligations or transections are made of either the L5 and L6 spinal nerves, or the L% spinal nerve only. The fourth variant involves an axotomy of two of the three terminal branches of the sciatic nerve (tibial and common peroneal nerves) leaving the remaining sural nerve intact whereas the last variant comprises the axotomy of only the tibial branch leaving the sural and common nerves uninjured. Control animals are treated with a sham operation.

Postoperatively, the nerve injured animals develop a chronic mechanical allodynia, cold allodynioa, as well as a thermal hyperalgesia. Mechanical allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, SA, USA; Electronic von Frey System, Somedic Sales AB, Hörby, Sweden). Thermal hyperalgesia is measured by means of a radiant heat source (Plantar Test, Ugo Basile, Comerio, Italy), or by means of a cold plate of 5 to 10 °C where the nocifensive reactions of the affected hind paw are counted as a measure of pain intensity. A further test for cold induced

pain is the counting of nocifensive reactions, or duration of nocifensive responses after plantar administration of acetone to the affected hind limb. Chronic pain in general is assessed by registering the circadian rhythms in activity (Surjo and Arndt, Universität zu Köln, Cologne, Germany), and by scoring differences in gait (foot print patterns; FOOTPRINTS program, Klapdor et al., 1997. A low cost method to analyze footprint patterns. J. Neurosci. Methods 75, 49-54).

Compounds are tested against sham operated and vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

Inflammatory Pain

Inflammatory pain is induced mainly in rats by injection of 0.75 mg carrageenan or complete Freund's adjuvant into one hind paw. The animals develop an edema with mechanical allodynia as well as thermal hyperalgesia. Mechanical allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, SA, USA). Thermal hyperalgesia is measured by means of a radiant heat source (Plantar Test, Ugo Basile, Comerio, Italy, Paw thermal stimulator, G. Ozaki, University of California, USA). For edema measurement two methods are being used. In the first method, the animals are sacrificed and the affected hindpaws sectioned and weighed. The second method comprises differences in paw volume by measuring water displacement in a plethysmometer (Ugo Basile, Comerio, Italy).

Compounds are tested against uninflamed as well as vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

Diabetic Neuropathic Pain

Rats treated with a single intraperitoneal injection of 50 to 80 mg/kg streptozotocin develop a profound hyperglycemia and mechanical allodynia within 1 to 3 weeks. Mechanical allodynia is measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC Inc.-Life Science Instruments, Woodland Hills, SA, USA).

Compounds are tested against diabetic and non-diabetic vehicle treated control groups. Substance application is performed at different time points via different application routes (i.v., i.p., p.o., i.t., i.c.v., s.c., intradermal, transdermal) prior to pain testing.

2. Parkinson's disease

6-Hydroxydopamine (6-OH-DA) Lesion

Degeneration of the dopaminergic nigrostriatal and striatopallidal pathways is the central pathological event in Parkinson's disease. This disorder has been mimicked experimentally in rats using single/sequential unilateral stereotaxic injections of 6-OH-DA into the medium forebrain bundle (MFB).

Male Wistar rats (Harlan Winkelmann, Germany), weighing 200±250 g at the beginning of the experiment, are used. The rats are maintained in a temperature- and humidity-controlled environment under a 12 h light/dark cycle with free access to food and water when not in experimental sessions. The following in vivo protocols are approved by the governmental authorities. All efforts are made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques.

Animals are administered pargyline on the day of surgery (Sigma, St. Louis, MO, USA; 50 mg/kg i.p.) in order to inhibit metabolism of 6-OHDA by monoamine oxidase and desmethylimipramine HCl (Sigma; 25 mg/kg i.p.) in order to prevent uptake of 6-OHDA by noradrenergic terminals. Thirty minutes later the rats are anesthetized with sodium pentobarbital (50 mg/kg) and placed in a stereotaxic frame. In order to lesion the DA nigrostriatal pathway 4 µl of 0.01% ascorbic acid-saline containing 8 µg of 6-OHDA HBr (Sigma) are injected into the left medial fore-brain bundle at a rate of 1 µl/min (2.4 mm anterior, 1.49 mm lateral, -2.7 mm ventral to Bregma and the skull surface). The needle is left in place an additional 5 min to allow diffusion to occur.

Stepping Test

Forelimb akinesia is assessed three weeks following lesion placement using a modified stepping test protocol. In brief, the animals are held by the experimenter with one hand fixing the hindlimbs and slightly raising the hind part above the surface. One paw is touching the table, and is then moved slowly sideways (5 s for 1 m), first in the forehand and then in the backhand direction. The number of adjusting steps is counted for both paws in the backhand and forehand direction of movement. The sequence of testing is right paw forehand and backhand adjusting stepping, followed by left paw forehand and backhand directions. The test is repeated three times on three consecutive days, after an initial training period of three days prior to the first testing. Forehand adjusted stepping reveals no consistent differences between lesioned and healthy control animals. Analysis is therefore restricted to backhand adjusted stepping.

Balance Test

Balance adjustments following postural challenge are also measured during the stepping test sessions. The rats are held in the same position as described in the

stepping test and, instead of being moved sideways, tilted by the experimenter towards the side of the paw touching the table. This maneuver results in loss of balance and the ability of the rats to regain balance by forelimb movements is scored on a scale ranging from 0 to 3. Score 0 is given for a normal forelimb placement. When the forelimb movement is delayed but recovery of postural balance detected, score 1 is given. Score 2 represents a clear, yet insufficient, forelimb reaction, as evidenced by muscle contraction, but lack of success in recovering balance, and score 3 is given for no reaction of movement. The test is repeated three times a day on each side for three consecutive days after an initial training period of three days prior to the first testing.

Staircase Test (Paw Reaching)

A modified version of the staircase test is used for evaluation of paw reaching behavior three weeks following primary and secondary lesion placement. Plexiglass test boxes with a central platform and a removable staircase on each side are used. The apparatus is designed such that only the paw on the same side at each staircase can be used, thus providing a measure of independent forelimb use. For each test the animals are left in the test boxes for 15 min. The double staircase is filled with 7 x 3 chow pellets (Precision food pellets, formula: P, purified rodent diet, size 45 mg; Sandown Scientific) on each side. After each test the number of pellets eaten (successfully retrieved pellets) and the number of pellets taken (touched but dropped) for each paw and the success rate (pellets eaten/pellets taken) are counted separately. After three days of food deprivation (12 g per animal per day) the animals are tested for 11 days. Full analysis is conducted only for the last five days.

MPTP treatment

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) causes degeneration of mesencephalic dopaminergic (DAergic) neurons in rodents, non-human

primates, and humans and, in so doing, reproduces many of the symptoms of Parkinson's disease. MPTP leads to a marked decrease in the levels of dopamine and its metabolites, and in the number of dopaminergic terminals in the striatum as well as severe loss of the tyrosine hydroxylase (TH)-immunoreactive cell bodies in the substantia nigra, pars compacta.

In order to obtain severe and long-lasting lesions, and to reduce mortality, animals receive single injections of MPTP, and are then tested for severity of lesion 7–10 days later. Successive MPTP injections are administered on days 1, 2 and 3. Animals receive application of 4 mg/kg MPTP hydrochloride (Sigma) in saline once daily. All injections are intraperitoneal (i.p.) and the MPTP stock solution is frozen between injections. Animals are decapitated on day 11.

Immunohistology

At the completion of behavioral experiments, all animals are anaesthetized with 3 ml thiopental (1 g/40 ml i.p., Tyrol Pharma). The mice are perfused transcardially with 0.01 M PBS (pH 7.4) for 2 min, followed by 4% paraformaldehyde (Merck) in PBS for 15 min. The brains are removed and placed in 4% paraformaldehyde for 24 h at 4 °C. For dehydration they are then transferred to a 20% sucrose (Merck) solution in 0.1 M PBS at 4 °C until they sink. The brains are frozen in methylbutan at -20 °C for 2 min and stored at -70 °C. Using a sledge microtome (mod. 3800-Frigocut, Leica), 25 µm sections are taken from the genu of the corpus callosum (AP 1.7 mm) to the hippocampus (AP 21.8 mm) and from AP 24.16 to AP 26.72. Forty-six sections are cut and stored in assorters in 0.25 M Tris buffer (pH 7.4) for immunohistochemistry.

A series of sections is processed for free-floating tyrosine hydroxylase (TH) immunohistochemistry. Following three rinses in 0.1 M PBS, endogenous peroxidase activity is quenched for 10 min in 0.3% H_2O_2 ±PBS. After rinsing in PBS, sections

are preincubated in 10% normal bovine serum (Sigma) for 5 min as blocking agent and transferred to either primary anti-rat TH rabbit antiserum (dilution 1:2000).

Following overnight incubation at room temperature, sections for TH immunoreactivity are rinsed in PBS (2 x10 min) and incubated in biotinylated anti-rabbit immunoglobulin G raised in goat (dilution 1:200) (Vector) for 90 min, rinsed repeatedly and transferred to Vectastain ABC (Vector) solution for 1 h. 3,.3' -Diaminobenzidine tetrahydrochloride (DAB; Sigma) in 0.1 M PBS, supplemented with 0.005% H_2O_2 , serves as chromogen in the subsequent visualization reaction. Sections are mounted on to gelatin-coated slides, left to dry overnight, counter-stained with hematoxylin dehydrated in ascending alcohol concentrations and cleared in butylacetate. Coverslips are mounted on entellan.

Rotarod Test

We use a modification of the procedure described by Rozas and Labandeira-Garcia (1997), with a CR-1 Rotamex system (Columbus Instruments, Columbus, OH) comprising an IBM-compatible personal computer, a CIO-24 data acquisition card, a control unit, and a four-lane rotarod unit. The rotarod unit consists of a rotating spindle (diameter 7.3 cm) and individual compartments for each mouse. The system software allows preprogramming of session protocols with varying rotational speeds (0–80 rpm). Infrared beams are used to detect when a mouse has fallen onto the base grid beneath the rotarod. The system logs the fall as the end of the experiment for that mouse, and the total time on the rotarod, as well as the time of the fall and all the set-up parameters, are recorded. The system also allows a weak current to be passed through the base grid, to aid training.

3. Dementia

The object recognition task

The object recognition task has been designed to assess the effects of experimental manipulations on the cognitive performance of rodents. A rat is placed in an open field, in which two identical objects are present. The rats inspects both objects during the first trial of the object recognition task. In a second trial, after a retention interval of for example 24 hours, one of the two objects used in the first trial, the 'familiar' object, and a novel object are placed in the open field. The inspection time at each of the objects is registered. The basic measures in the OR task is the time spent by a rat exploring the two object the second trial. Good retention is reflected by higher exploration times towards the novel than the 'familiar' object.

Administration of the putative cognition enhancer prior to the first trial predominantly allows assessment of the effects on acquisition, and eventually on consolidation processes. Administration of the testing compound after the first trial allows to assess the effects on consolidation processes, whereas administration before the second trial allows to measure effects on retrieval processes.

The passive avoidance task

The passive avoidance task assesses memory performance in rats and mice. The inhibitory avoidance apparatus consists of a two-compartment box with a light compartment and a dark compartment. The two compartments are separated by a guillotine door that can be operated by the experimenter. A threshold of 2 cm separates the two compartments when the guillotine door is raised. When the door is open, the illumination in the dark compartment is about 2 lux. The light intensity is about 500 lux at the center of the floor of the light compartment.

Two habituation sessions, one shock session, and a retention session are given, separated by inter-session intervals of 24 hours. In the habituation sessions and the retention session the rat is allowed to explore the apparatus for 300 sec. The rat is placed in the light compartment, facing the wall opposite to the guillotine door. After an accommodation period of 15 sec. the guillotine door is opened so that all parts of the apparatus can be visited freely. Rats normally avoid brightly lit areas and will enter the dark compartment within a few seconds.

In the shock session the guillotine door between the compartments is lowered as soon as the rat has entered the dark compartment with its four paws, and a scrambled 1 mA footshock is administered for 2 sec. The rat is removed from the apparatus and put back into its home cage. The procedure during the retention session is identical to that of the habituation sessions.

The step-through latency, that is the first latency of entering the dark compartment (in sec.) during the retention session is an index of the memory performance of the animal; the longer the latency to enter the dark compartment, the better the retention is. A testing compound in given half an hour before the shock session, together with 1 $mg*kg^{-1}$ scopolamine. Scopolamine impairs the memory performance during the retention session 24 hours later. If the test compound increases the enter latency compared with the scopolamine-treated controls, is likely to possess cognition enhancing potential.

The Morris water escape task

The Morris water escape task measures spatial orientation learning in rodents. It is a test system that has extensively been used to investigate the effects of putative therapeutic on the cognitive functions of rats and mice. The performance of an animal is assessed in a circular water tank with an escape platform that is submerged about 1 cm below the surface of the water. The escape platform is not visible for an

animal swimming in the water tank. Abundant extra-maze cues are provided by the furniture in the room, including desks, computer equipment, a second water tank, the presence of the experimenter, and by a radio on a shelf that is playing softly.

The animals receive four trials during five daily acquisition sessions. A trial is started by placing an animal into the pool, facing the wall of the tank. Each of four starting positions in the quadrants north, east, south, and west is used once in a series of four trials; their order is randomized. The escape platform is always in the same position. A trial is terminated as soon as the animal had climbs onto the escape platform or when 90 seconds have elapsed, whichever event occurs first. The animal is allowed to stay on the platform for 30 seconds. Then it is taken from the platform and the next trial is started. If an animal did not find the platform within 90 seconds it is put on the platform by the experimenter and is allowed to stay there for 30 seconds. After the fourth trial of the fifth daily session, an additional trial is given as a probe trial: the platform is removed, and the time the animal spends in the four quadrants is measured for 30 or 60 seconds. In the probe trial, all animals start from the same start position, opposite to the quadrant where the escape platform had been positioned during acquisition.

Four different measures are taken to evaluate the performance of an animal during acquisition training: escape latency, traveled distance, distance to platform, and swimming speed. The following measures are evaluated for the probe trial: time (s) in quadrants and traveled distance (cm) in the four quadrants. The probe trial provides additional information about how well an animal learned the position of the escape platform. If an animal spends more time and swims a longer distance in the quadrant where the platform had been positioned during the acquisition sessions than in any other quadrant, one concludes that the platform position has been learned well.

In order to assess the effects of putative cognition enhancing compounds, rats or mice with specific brain lesions which impair cognitive functions, or animals treated

with compounds such as scopolamine or MK-801, which interfere with normal learning, or aged animals which suffer from cognitive deficits, are used.

The T-maze spontaneous alternation task

The T-maze spontaneous alternation task (TeMCAT) assesses the spatial memory performance in mice. The start arm and the two goal arms of the T-maze are provided with guillotine doors which can be operated manually by the experimenter. A mouse is put into the start arm at the beginning of training. The guillotine door is closed. In the first trial, the 'forced trial', either the left or right goal arm is blocked by lowering the guillotine door. After the mouse has been released from the start arm, it will negotiate the maze, eventually enter the open goal arm, and return to the start position, where it will be confined for 5 seconds, by lowering the guillotine door. Then, the animal can choose freely between the left and right goal arm (all guillotine-doors opened) during 14 'free choice' trials. As soon a the mouse has entered one goal arm, the other one is closed. The mouse eventually returns to the start arm and is free to visit whichever go alarm it wants after having been confined to the start arm for 5 seconds. After completion of 14 free choice trials in one session, the animal is removed from the maze. During training, the animal is never handled.

The per-cent alternations out of 14 trials is calculated. This percentage and the total time needed to complete the first forced trial and the subsequent 14 free choice trials (in s) is analyzed. Cognitive deficits are usually induced by an injection of scopolamine, 30 min before the start of the training session. Scopolamine reduced the per-cent alternations to chance level, or below. A cognition enhancer, which is always administered before the training session, will at least partially, antagonize the scopolamine-induced reduction in the spontaneous alternation rate.

EXAMPLE 16

Diabetes: In vivo testing of compounds/target validation

1. Glucose Production:

Over-production of glucose by the liver, due to an enhanced rate of gluconeogenesis, is the major cause of fasting hyperglycemia in diabetes. Overnight fasted normal rats or mice have elevated rates of gluconeogenesis as do streptozotocin-induced diabetic rats or mice fed ad libitum. Rats are made diabetic with a single intravenous injection of 40 mg/kg of streptozotocin while C57BL/KsJ mice are given 40-60 mg/kg i.p. for 5 consecutive days. Blood glucose is measured from tail-tip blood and then compounds are administered via different routes (p.o., i.p., i.v., s.c.). Blood is collected at various times thereafter and glucose measured. Alternatively, compounds are administered for several days, then the animals are fasted overnight, blood is collected and plasma glucose measured. Compounds that inhibit glucose production will decrease plasma glucose levels compared to the vehicle-treated control group.

2. Insulin Sensitivity:

Both ob/ob and db/db mice as well as diabetic Zucker rats are hyperglycemic, hyperinsulinemic and insulin resistant. The animals are pre-bled, their glucose levels measured, and then they are grouped so that the mean glucose level is the same for each group. Compounds are administered daily either q.d. or b.i.d. by different routes (p.o., i.p., s.c.) for 7-28 days. Blood is collected at various times and plasma glucose and insulin levels determined. Compounds that improve insulin sensitivity in these models will decrease both plasma glucose and insulin levels when compared to the vehicle-treated control group.

3. Insulin Secretion:

Compounds that enhance insulin secretion from the pancreas will increase plasma insulin levels and improve the disappearance of plasma glucose following the administration of a glucose load. When measuring insulin levels, compounds are administered by different routes (p.o., i.p., s.c. or i.v.) to overnight fasted normal rats or mice. At the appropriate time an intravenous glucose load (0.4g/kg) is given, blood is collected one minute later. Plasma insulin levels are determined. Compounds that enhance insulin secretion will increase plasma insulin levels compared to animals given only glucose. When measuring glucose disappearance, animals are bled at the appropriate time after compound administration, then given either an oral or intraperitoneal glucose load (1g/kg), bled again after 15, 30, 60 and 90 minutes and plasma glucose levels determined. Compounds that increase insulin levels will decrease glucose levels and the area-under-the glucose curve when compared to the vehicle-treated group given only glucose.

Compounds that enhance insulin secretion from the pancreas will increase plasma insulin levels and improve the disappearance of plasma glucose following the administration of a glucose load. When measuring insulin levels, test compounds which regulate 5-HT-like GPCR are administered by different routes (p.o., i.p., s.c., or i.v.) to overnight fasted normal rats or mice. At the appropriate time an intravenous glucose load (0.4g/kg) is given, blood is collected one minute later. Plasma insulin levels are determined. Test compounds that enhance insulin secretion will increase plasma insulin levels compared to animals given only glucose. When measuring glucose disappearance, animals are bled at the appropriate time after compound administration, then given either an oral or intraperitoneal glucose load (1g/kg), bled again after 15, 30, 60, and 90 minutes and plasma glucose levels determined. Test compounds that increase insulin levels will decrease glucose levels and the area-under-the glucose curve when compared to the vehicle-treated group given only glucose.

4. Glucose Production:

Over-production of glucose by the liver, due to an enhanced rate of gluconeogenesis, is the major cause of fasting hyperglycemia in diabetes. Overnight fasted normal rats or mice have elevated rates of gluconeogenesis as do streptozotocin-induced diabetic rats or mice fed ad libitum. Rats are made diabetic with a single intravenous injection of 40 mg/kg of streptozotocin while C57BL/KsJ mice are given 40-60 mg/kg i.p. for 5 consecutive days. Blood glucose is measured from tail-tip blood and then compounds are administered via different routes (p.o., i.p., i.v., s.c.). Blood is collected at various times thereafter and glucose measured. Alternatively, compounds are administered for several days, then the animals are fasted overnight, blood is collected and plasma glucose measured. Compounds that inhibit glucose production will decrease plasma glucose levels compared to the vehicle-treated control group.

5. Insulin Sensitivity:

Both ob/ob and db/db mice as well as diabetic Zucker rats are hyperglycemic, hyperinsulinemic and insulin resistant. The animals are pre-bled, their glucose levels measured, and then they are grouped so that the mean glucose level is the same for each group. Compounds are administered daily either q.d. or b.i.d. by different routes (p.o., i.p., s.c.) for 7-28 days. Blood is collected at various times and plasma glucose and insulin levels determined. Compounds that improve insulin sensitivity in these models will decrease both plasma glucose and insulin levels when compared to the vehicle-treated control group.

6. Insulin Secretion:

Compounds that enhance insulin secretion from the pancreas will increase plasma insulin levels and improve the disappearance of plasma glucose following the administration of a glucose load. When measuring insulin levels, compounds are administered by different routes (p.o., i.p., s.c. or i.v.) to overnight fasted normal rats or mice. At the appropriate time an intravenous glucose load (0.4g/kg) is given, blood is collected one minute later. Plasma insulin levels are determined. Compounds that enhance insulin secretion will increase plasma insulin levels compared to animals given only glucose. When measuring glucose disappearance, animals are bled at the appropriate time after compound administration, then given either an oral or intraperitoneal glucose load (1g/kg), bled again after 15, 30, 60 and 90 minutes and plasma glucose levels determined. Compounds that increase insulin levels will decrease glucose levels and the area-under-the glucose curve when compared to the vehicle-treated group given only glucose.

EXAMPLE 17

Proliferation inhibition assay: Antisense oligonucleotides suppress the growth of cancer cell lines

The cell line used for testing is the human colon cancer cell line HCT116. Cells are cultured in RPMI-1640 with 10-15% fetal calf serum at a concentration of 10,000 cells per milliliter in a volume of 0.5 ml and kept at 37 °C in a 95% air/5%CO_2 atmosphere.

Phosphorothioate oligoribonucleotides are synthesized on an Applied Biosystems Model 380B DNA synthesizer using phosphoroamidite chemistry. A sequence of 24 bases complementary to the nucleotides at position 1 to 24 of SEQ ID NO:1 is used as the test oligonucleotide. As a control, another (random) sequence is used: 5'-TCA ACT GAC TAG ATG TAC ATG GAC-3'. Following assembly and deprotection,

oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate buffered saline at the desired concentration. Purity of the oligonucleotides is tested by capillary gel electrophoresis and ion exchange HPLC. The purified oligonucleotides are added to the culture medium at a concentration of 10 μM once per day for seven days.

The addition of the test oligonucleotide for seven days results in significantly reduced expression of human 5-HT-like GPCR as determined by Western blotting. This effect is not observed with the control oligonucleotide. After 3 to 7 days, the number of cells in the cultures is counted using an automatic cell counter. The number of cells in cultures treated with the test oligonucleotide (expressed as 100%) is compared with the number of cells in cultures treated with the control oligonucleotide. The number of cells in cultures treated with the test oligonucleotide is not more than 30% of control, indicating that the inhibition of human 5-HT-like GPCR has an anti-proliferative effect on cancer cells.

EXAMPLE 18

In vivo testing of compounds/target validation

1. Acute Mechanistic Assays

1.1. Reduction in Mitogenic Plasma Hormone Levels

This non-tumor assay measures the ability of a compound to reduce either the endogenous level of a circulating hormone or the level of hormone produced in response to a biologic stimulus. Rodents are administered test compound (p.o., i.p., i.v., i.m., or s.c.). At a predetermined time after administration of test compound, blood plasma is collected. Plasma is assayed for levels of the hormone of interest. If the normal circulating levels of the hormone are too low and/or variable to provide consistent results, the level of the hormone may be elevated by a pre-treatment with a biologic stimulus (i.e., LHRH may be injected i.m. into mice at a dosage of 30 ng/mouse to induce a burst of testosterone synthesis). The timing of plasma

collection would be adjusted to coincide with the peak of the induced hormone response. Compound effects are compared to a vehicle-treated control group. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test. Significance is p value $\leq$ 0.05 compared to the vehicle control group.

1.2. Hollow Fiber Mechanism of Action Assay

Hollow fibers are prepared with desired cell line(s) and implanted intraperitoneally and/or subcutaneously in rodents. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Fibers are harvested in accordance with specific readout assay protocol, these may include assays for gene expression (bDNA, PCR, or Taqman), or a specific biochemical activity (i.e., cAMP levels. Results are analyzed by Student's t-test or Rank Sum test after the variance between groups is compared by an F-test, with significance at p $\leq$ 0.05 as compared to the vehicle control group.

2. Subacute Functional *In Vivo* Assays

2.1. Reduction in Mass of Hormone Dependent Tissues

This is another non-tumor assay that measures the ability of a compound to reduce the mass of a hormone dependent tissue (i.e., seminal vesicles in males and uteri in females). Rodents are administered test compound (p.o., i.p., i.v., i.m., or s.c.) according to a predetermined schedule and for a predetermined duration (i.e., 1 week). At termination of the study, animals are weighed, the target organ is excised, any fluid is expressed, and the weight of the organ is recorded. Blood plasma may also be collected. Plasma may be assayed for levels of a hormone of interest or for levels of test agent. Organ weights may be directly compared or they may be normalized for the body weight of the animal. Compound effects are compared to a vehicle-treated control group. An F-test is preformed to determine if the variance is

equal or unequal followed by a Student's t-test. Significance is p value $\leq$ 0.05 compared to the vehicle control group.

2.2. Hollow Fiber Proliferation Assay

Hollow fibers are prepared with desired cell line(s) and implanted intraperitoneally and/or subcutaneously in rodents. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Fibers are harvested in accordance with specific readout assay protocol. Cell proliferation is determined by measuring a marker of cell number (i.e., MTT or LDH). The cell number and change in cell number from the starting inoculum are analyzed by Student's t-test or Rank Sum test after the variance between groups is compared by an F-test, with significance at $p \leq 0.05$ as compared to the vehicle control group.

2.3. Anti-angiogenesis Models

2.3.1. Corneal Angiogenesis

Hydron pellets with or without growth factors or cells are implanted into a micropocket surgically created in the rodent cornea. Compound administration may be systemic or local (compound mixed with growth factors in the hydron pellet). Corneas are harvested at 7 days post implantation immediately following intracardiac infusion of colloidal carbon and are fixed in 10% formalin. Readout is qualitative scoring and/or image analysis. Qualitative scores are compared by Rank Sum test. Image analysis data is evaluated by measuring the area of neovascularization (in pixels) and group averages are compared by Student's t-test (2 tail). Significance is $p \leq 0.05$ as compared to the growth factor or cells only group.

2.3.2. Matrigel Angiogenesis

Matrigel, containing cells or growth factors, is injected subcutaneously. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Matrigel plugs are harvested at predetermined time point(s) and prepared for readout. Readout is an ELISA-based assay for hemoglobin concentration and/or histological examination (i.e. vessel count, special staining for endothelial surface markers: CD31, factor-8). Readouts are analyzed by Student's t-test, after the variance between groups is compared by an F-test, with significance determined at $p \leq 0.05$ as compared to the vehicle control group.

3. Primary Antitumor Efficacy

3.1. Early Therapy Models

3.1.1. Subcutaneous Tumor

Tumor cells or fragments are implanted subcutaneously on Day 0. Vehicle and/or compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule starting at a time, usually on Day 1, prior to the ability to measure the tumor burden. Body weights and tumor measurements are recorded 2-3 times weekly. Mean net body and tumor weights are calculated for each data collection day. Antitumor efficacy may be initially determined by comparing the size of treated (T) and control (C) tumors on a given day by a Student's t-test, after the variance between groups is compared by an F-test, with significance determined at $p \leq 0.05$. The experiment may also be continued past the end of dosing in which case tumor measurements would continue to be recorded to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is $p \leq 0.05$.

3.1.2. Intraperitoneal/Intracranial Tumor Models

Tumor cells are injected intraperitoneally or intracranially on Day 0. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule starting on Day 1. Observations of morbidity and/or mortality are recorded twice daily. Body weights are measured and recorded twice weekly. Morbidity/mortality data is expressed in terms of the median time of survival and the number of long-term survivors is indicated separately. Survival times are used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment.

3.2. Established Disease Model

Tumor cells or fragments are implanted subcutaneously and grown to the desired size for treatment to begin. Once at the predetermined size range, mice are randomized into treatment groups. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Tumor and body weights are measured and recorded 2-3 times weekly. Mean tumor weights of all groups over days post inoculation are graphed for comparison. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. Tumor measurements may be recorded after dosing has stopped to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is p value$\leq$ 0.05 compared to the vehicle control group.

3.3. Orthotopic Disease Models

3.3.1. Mammary Fat Pad Assay

Tumor cells or fragments, of mammary adenocarcinoma origin, are implanted directly into a surgically exposed and reflected mammary fat pad in rodents. The fat pad is placed back in its original position and the surgical site is closed. Hormones may also be administered to the rodents to support the growth of the tumors. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Tumor and body weights are measured and recorded 2-3 times weekly. Mean tumor weights of all groups over days post inoculation are graphed for comparison. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group.

Tumor measurements may be recorded after dosing has stopped to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is p value≤ 0.05 compared to the vehicle control group. In addition, this model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ, or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.2. Intraprostatic Assay

Tumor cells or fragments, of prostatic adenocarcinoma origin, are implanted directly into a surgically exposed dorsal lobe of the prostate in rodents. The prostate is externalized through an abdominal incision so that the tumor can be implanted specifically in the dorsal lobe while verifying that the implant does not enter the seminal vesicles. The successfully inoculated prostate is replaced in the abdomen and the incisions through the abdomen and skin are closed. Hormones may also be administered to the rodents to support the growth of the tumors. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the lungs), or measuring the target organ weight (i.e., the regional lymph nodes). The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.3. Intrabronchial Assay

Tumor cells of pulmonary origin may be implanted intrabronchially by making an incision through the skin and exposing the trachea. The trachea is pierced with the beveled end of a 25 gauge needle and the tumor cells are inoculated into the main bronchus using a flat-ended 27 gauge needle with a 90° bend. Compounds are

administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the contralateral lung), or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.4. Intracecal Assay

Tumor cells of gastrointestinal origin may be implanted intracecally by making an abdominal incision through the skin and externalizing the intestine. Tumor cells are inoculated into the cecal wall without penetrating the lumen of the intestine using a 27 or 30 gauge needle. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the liver), or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test,

with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

4. Secondary (Metastatic) Antitumor Efficacy

4.1. Spontaneous Metastasis

Tumor cells are inoculated s.c. and the tumors allowed to grow to a predetermined range for spontaneous metastasis studies to the lung or liver. These primary tumors are then excised. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule which may include the period leading up to the excision of the primary tumor to evaluate therapies directed at inhibiting the early stages of tumor metastasis. Observations of morbidity and/or mortality are recorded daily. Body weights are measured and recorded twice weekly. Potential endpoints include survival time, numbers of visible foci per target organ, or target organ weight. When survival time is used as the endpoint the other values are not determined. Survival data is used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment. The mean number of visible tumor foci, as determined under a dissecting microscope, and the mean target organ weights are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment for both of these endpoints.

4.2. Forced Metastasis

Tumor cells are injected into the tail vein, portal vein, or the left ventricle of the heart in experimental (forced) lung, liver, and bone metastasis studies, respectively. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Observations of morbidity and/or mortality are recorded daily. Body weights are measured and recorded twice weekly. Potential endpoints include survival time, numbers of visible foci per target organ, or target organ weight. When

survival time is used as the endpoint the other values are not determined. Survival data is used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment. The mean number of visible tumor foci, as determined under a dissecting microscope, and the mean target organ weights are compared by Student's t-test after conducting an F-test, with significance at $p \leq 0.05$ compared to the vehicle control group in the experiment for both endpoints.

EXAMPLE 19

Screening for G_i -, G_s-, and G_q- coupled receptors

G_i-coupled receptors. Cells are stably transfected with the relevant receptor and with an inducible CRE-luciferase construct. Cells are grown in 50% Dulbecco's modified Eagle medium / 50% F12 (DMEM/F12) supplemented with 10% FBS, at 37°C in a humidified atmosphere with 10% CO_2 and are routinely split at a ratio of 1:10 every 2 or 3 days. Test cultures are seeded into 384 – well plates at an appropriate density (e.g. 2000 cells / well in 35 µl cell culture medium) in DMEM/F12 with FBS, and are grown for 48 hours (range: ~ 24 - 60 hours, depending on cell line). Growth medium is then exchanged against serum free medium (SFM; e.g. Ultra-CHO), containing 0.1% BSA.

Test compounds dissolved in DMSO are diluted in SFM and transferred to the test cultures (maximal final concentration 10 µmolar), followed by addition of forskolin (~ 1 µmolar, final conc.) in SFM + 0,1% BSA 10 minutes later. In case of antagonist screening both, an appropriate concentration of agonist, and forskolin are added. The plates are incubated at 37°C in 10% CO_2 for 3 hours. Then the supernatant is removed, cells are lysed with lysis reagent (25 mmolar phosphate-buffer, pH 7,8 , containing 2 mmolar DDT, 10% glycerol and 3% Triton X100).

The luciferase reaction is started by addition of substrate-buffer (e.g. luciferase assay reagent, Promega) and luminescence is immediately determined (e.g. Berthold luminometer or Hamamatzu camera system).

G_s -coupled receptors. Cells are stably transfected with the relevant receptor and with an inducible CRE-luciferase construct. Cells are grown in 50% Dulbecco's modified Eagle medium / 50% F12 (DMEM/F12) supplemented with 10% FBS, at 37°C in a humidified atmosphere with 10% CO_2 and are routinely split at a ratio of 1:10 every 2 or 3 days. Test cultures are seeded into 384 – well plates at an appropriate density (e.g. 1000 or 2000 cells / well in 35 µl cell culture medium) in DMEM/F12 with FBS, and are grown for 48 hours (range: ~ 24 - 60 hours, depending on cell line). The assay is started by addition of test-compounds in serum free medium (SFM; e.g. Ultra-CHO) containing 0.1% BSA.

Test compounds are dissolved in DMSO, diluted in SFM and transferred to the test cultures (maximal final concentration 10 µmolar, DMSO conc. < 0,6 %). In case of antagonist screening an appropriate concentration of agonist is added 5 – 10 minutes later. The plates are incubated at 37°C in 10% CO_2 for 3 hours. Then the cells are lysed with 10 µl lysis reagent per well (25 mmolar phosphate-buffer, pH 7,8 , containing 2 mmolar DDT, 10% glycerol and 3% Triton X100).

The luciferase reaction is started by addition of 20 µl substrate-buffer per well (e.g. luciferase assay reagent, Promega). Measurement of luminescence is started immediately (e.g. Berthold luminometer or Hamamatzu camera system).

G_q -coupled receptors. Cells are stably transfected with the relevant receptor. Cells expressing functional receptor protein are grown in 50% Dulbecco's modified Eagle medium / 50% F12 (DMEM/F12) supplemented with 10% FBS, at 37°C in a humidified atmosphere with 5% CO_2 and are routinely split at a cell line dependent ratio every 3 or 4 days. Test cultures are seeded into 384 – well plates at an

appropriate density (e.g. 2000 cells / well in 35 µl cell culture medium) in DMEM/F12 with FBS, and are grown for 48 hours (range: ~ 24 - 60 hours, depending on cell line). Growth medium is then exchanged against physiological salt solution (e.g. Tyrode solution).

Test compounds dissolved in DMSO are diluted in Tyrode solution containing 0.1% BSA and transferred to the test cultures (maximal final concentration 10 µmolar). After addition of the receptor specific agonist the resulting Gq-mediated intracellular calcium increase is measured using appropriate read-out systems (e.g. calcium-sensitive dyes).

EXAMPLE 20

Promoter assay

A promoter assay is set up with a human hepatocellular carcinoma cell HepG2 that was stably transfected with a luciferase gene under the control of a serotonin-regulated promoter. The vector 2xIROluc, which is used for transfection, carries a serotonin responsive element of two 12 bp inverted palindromes separated by an 8 bp spacer in front of a tk minimal promoter and the luciferase gene.

Test cultures are seeded in 96 well plates in serum - free Eagle's Minimal Essential Medium supplemented with glutamine, tricine, sodium pyruvate, non – essential amino acids, insulin, selen, transferrin, and are cultivated in a humidified atmosphere at 10 % CO_2 at 37°C. After 48 hours of incubation, serial dilutions of test compounds or reference compounds and costimulator if appropriate (final concentration 1 nM) are added to the cell cultures and incubation is continued for the optimal time (e.g. another 4-72 hours). The cells are then lysed by addition of buffer containing Triton X100 and luciferin and the luminescence of luciferase induced by test or reference compounds is measured in a luminometer. For each concentration of a test compound

replicates of 4 are tested. EC_{50} – values for each test compound are calculated by use of the Graph Pad Prism Scientific software.

EXAMPLE 21

Expression profiling

Total cellular RNA was isolated from cells by one of two standard methods: (1) guanidine isothiocyanate/Cesium chloride density gradient centrifugation or (2) the Tri-Reagent protocol according to the manufacturer's specifications (Molecular Research Center, Inc., Cincinatti, Ohio). Total RNA prepared by the Tri-reagent protocol was treated with DNAse I to remove genomic DNA contamination. RNA was prepared from the following sources: coronary smooth muscle cells, brain, testis, pancreas, stomach, cerebellum, trachea, adrenal gland, skeletal muscle, salivary gland, small intestine, prostate, fetal liver, placenta, fetal brain, uterus, mammary gland, heart, spleen, lung, HeLa cells, liver, kidney, thymus, bone marrow, thyroid, colon, bladder, spinal cord, peripheral blood, liver cirrhosis, pancreas liver cirrhosis, spleen liver cirrhosis, total Alzheimer brain, fetal lung, breast tumor, colon tumor, lung tumor, HEK 293 cells, adipose, pericardium, fetal heart, thyroid tumor, MDA MB 231 cells, HEP G2 cells, HUVEC cells, fetal kidney, breast, Jurkat T-cells, Alzheimer brain cortex, cervix, esophagus, thalamus, precentral gyrus, hippocampus, occipital lobe, cerebral peduncles, postcentral gyrus, temporal lobe, parietal lobe, cerebellum (right), cerebellum (left), tonsilla cerebelli, cerebral meninges, pons, frontal lobe, cerebral cortex, corpus callosum, vermis cerebelli, Alzheimer brain frontal lobe, interventricular septum, heart atrium (right), heart atrium (left), heart ventricle (left), skin, ileum chronic inflammation, erythrocytes, aorta, lung COPD, aorta sclerotic, prostate BPH, rectum, retina, penis, artery, vein, lymph node, dorsal root ganglia, corpus cavernosum, and thrombocytes.

For relative quantitation of the mRNA distribution of serotonin-like GPCR, total RNA from each cell or tissue source was first reverse transcribed. Eighty-five µg of

total RNA was reverse transcribed using 1 µmole random hexamer primers, 0.5 mM each of dATP, dCTP, dGTP, and dTTP (Qiagen, Hilden, Germany), 3000 U RnaseQut (Invitrogen, Groningen, Netherlands) in a final volume of 680 µl. The first strand synthesis buffer and Omniscript (2 u/µl) reverse transcriptase were from (Qiagen, Hilden, Germany). The reaction was incubated at 37 °C for 90 minutes and cooled on ice. The volume was adjusted to 6800 µl with water, yielding a final concentration of 12.5 ng/µl of starting RNA.

For relative quantitation of the distribution of serotonin-like GPCR mRNA in cells and tissues the Perkin Elmer ABI Prism®. 7700 Sequence Detection system or Biorad iCycler was used according to the manufacturer's specifications and protocols. PCR reactions were set up to quantitate X and the housekeeping genes HPRT, GAPDH, beta-actin, and others. Forward and reverse primers and probe for the serotonin-like GPCR were designed using the Perkin Elmer ABI Primer Express™. software and were synthesized by TibMolBiol (Berlin, Germany). The X forward primer sequence was: GTCCTTCACCGCCATTGTG (SEQ ID NO: 5). The X reverse primer sequence was TGCACACCTACCTGGCAGTCATCCA (SEQ ID NO:6). The fluorogenic probe, labeled with FAM as the reporter dye and TAMRA as the quencher, is AAGGAGAGGTAGCGCAGTGG (SEQ ID NO: 7).

The following reactions in a final volume of 25 µl were set up: 1X TaqMan buffer A, 5.5 mM MgC12, 200 nM each of dATP, dCTP, dGTP, and dUTP, 0.025 U/µl AmpliTaq Gold™, 0.01 U/ µl AmpErase UNG® and probe 1X, serotonin-like GPCR forward and reverse primers each at 200 nM, 200 nM serotonin-like GPCR FAM/TAMRA-labeled probe, and 5 µl of template cDNA. Thermal cycling parameters were 2 min HOLD at 50 °C, 10 min HOLD at 95 °C, followed by melting at 95 °C for 15 sec and annealing/extending at 60 °C for 1 min for each of 40 cycles.

Calculation of corrected CT values

The CT-value is calculated as described above. The CF-value is calculated as followed :

1. PCR reactions were set up to quantitate the housekeeping genes (HKG) for each cDNA sample.
2. CT_{HKG}-values were calculated as described above
3. CT-mean values of all HKG for each cDNA are calculated (n = number of HKG):
   (CT_{HKG1}-value + CT_{HKG2}-value + CT_{HKG-X}-value) / n = CT_{cDNA-X}-mean values (n = number of HKG)
4. (CT_{cDNA-1}-mean value + CT_{cDNA-X}-mean value) / y = CT_{pannel}-mean value (y = number of cDNAs)
5. CT_{pannel}-mean value - CT_{cDNA-X}-mean value = CF_{cDNA-X}
6. $CT_{cDNA-X} + CF_{cDNA-X} = CT_{cor-cDNA-X}$

Calculation of relative expression

Definition : highest $CT_{cor-cDNA-X} \neq 40$ is defined as $CT_{cor-cDNA-X}$ [high]

Relative Expression = $2e(CT_{cor-cDNA-X}$ [high] - $CT_{cor-cDNA-Y})$

The results are shown in Figs. 11-13. The human serotonin-like GPCR is expressed in different human tissues. Serotonin-like GPCR mRNA is highly expressed in cerebellum, postcentral gyrus, dorsal root ganglia, erythrocyte, lung COPD, esophagus, ileum chronic inflammation, prostate BPH, and penis.

Serotonin-like GPCR mRNA is highly expressed in different brain tissues as cerebellum, postcentral gyrus and dorsal root ganglia. The expression in the above

mentioned tissues suggests an association between the human serotonin-like GPCR and peripheral and central nervous system diseases.

Serotonin-like GPCR mRNA is highly expressed in prostate BPH and penis. The expression in the above mentioned tissues suggests an association the human serotonin-like GPCR and genito-urinary diseases.

Serotonin-like GPCR mRNA is highly expressed in lung COPD. The expression in the above mentioned tissues suggests an association between the human serotonin-like GPCR and respiratory diseases.

Serotonin-like GPCR mRNA is highly expressed in erythrocytes. The expression in the above mentioned tissues suggests an association between the human serotonin-like GPCR and hematological diseases.

EXAMPLE 22

Measurement of rhythmic isovolumetric bladder contraction in anethetized rats

(1) Animals

Female Sprague-Dawley rats (200~250 g / Charles River Japan) are used.

(2) Catheter implantation

Rats are anesthetized by intraperitoneal administration of urethane (Sigma) at 1.25 g/kg. The abdomen is opened through a midline incision, and a polyethylene catheter (BECTON DICKINSON, PE50) is implanted into the bladder through the dome. In parallel, the inguinal region is incised, and a polyethylene catheter (BECTON DICKINSON, PE50) filled with saline (Otsuka) is inserted into a femoral vein.

(3) Investigation of bladder contraction

The bladder is filled via the catheter by incremental volume of saline until spontaneous bladder contractions occurred. The intravesical pressure is measured a pressure transducer and displayed continuously on a chart recorder. The activity of

test compounds is assessed after intraveneous administration through a polyethylene cannula inserted into the femoral vein.

Measurement of bladder cystometry in conscious rats

(1) Animals

Female Sprague-Dawley rats (200~250 g / Charles River Japan) are used.

(2) Catheter implantation

Rats are anesthetized by intramuscular administration of ketamine (75 mg/kg) and xylazine (15 mg/kg). The abdomen is opened through a midline incision, and a polyethylene catheter (BECTON DICKINSON, PE50) is implanted into the bladder through the dome. The catheter is tunneled through subcutis of the animal by needle (14G) to neck. In parallel, the inguinal region is incised, and a polyethylene catheter (BECTON DICKINSON, PE50) filled with saline (Otsuka) is inserted into a femoral vein. The catheter is tunneled through subcutis of the animal by needle to neck.

(3) Cystometric investigation

The bladder catheter is connected via T-tube to a pressure transducer (Viggo-Spectramed Pte Ltd, DT-XXAD) and a microinjection pump (TERUMO). Saline is infused at room temperature into the bladder at a rate of 10 ml/hr. Intravesical pressure is recorded continuously on a chart pen recorder (Yokogawa). At least three reproducible micturition cycles are recorded before a test compound administration.

(4) Administration of test compounds

A testing compound dissolved in the mixture of ethanol, Tween 80 (ICN Biomedicals Inc.) and saline (1 : 1 : 8, v/v/v) is administered intraveneously through the catheter.

REFERENCES

1. Human serotonin 1D receptor is encoded by a subfamily of two distinct genes: 5-HT1D alpha and 5-HT1D beta. Proc Natl Acad Sci U S A 1992 Apr 15;89(8):3630-4

2. Cloning and expression of the human 5-HT1B-type receptor gene. Biochem Biophys Res Commun 1992 Jun 15;185(2):517-23

3. Molecular cloning of a human serotonin receptor (S12) with a pharmacological profile resembling that of the 5-HT1D subtype. J Biol Chem 1992 Apr 15;267(11):7553-62

4. Human serotonin1B receptor expression in Sf9 cells: phosphorylation, palmitoylation, and adenylyl cyclase inhibition. Biochemistry 1993 Nov 2;32(43):11727-33

CLAIMS

1. An isolated polynucleotide being selected from the group consisting of:

a) a polynucleotide encoding a serotonin-like G protein-coupled receptor polypeptide comprising an amino acid sequence selected form the group consisting of:

amino acid sequences which are at least about 27% identical to

the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

b) a polynucleotide comprising the sequence of SEQ ID NO: 1;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b) and encodes a serotonin-like G protein-coupled receptor polypeptide;

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code and encodes a serotonin-like G protein-coupled receptor polypeptide; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d) and encodes a serotonin-like G protein-coupled receptor polypeptide.

2. An expression vector containing any polynucleotide of claim 1.

3. A host cell containing the expression vector of claim 2.

4. A substantially purified serotonin-like G protein-coupled receptor polypeptide encoded by a polynucleotide of claim 1.

5. A method for producing a serotonin-like G protein-coupled receptor polypeptide, wherein the method comprises the following steps:

a) culturing the host cell of claim 3 under conditions suitable for the expression of the serotonin-like G protein-coupled receptor polypeptide; and

b) recovering the serotonin-like G protein-coupled receptor polypeptide from the host cell culture.

6. A method for detection of a polynucleotide encoding a serotonin-like G protein-coupled receptor polypeptide in a biological sample comprising the following steps:

a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b) detecting said hybridization complex.

7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.

8. A method for the detection of a polynucleotide of claim 1 or a serotonin-like G protein-coupled receptor polypeptide of claim 4 comprising the steps of:
contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the serotonin-like G protein-coupled receptor polypeptide.

9. A diagnostic kit for conducting the method of any one of claims 6 to 8.

10. A method of screening for agents which decrease the activity of a serotonin-like G protein-coupled receptor, comprising the steps of:
contacting a test compound with any serotonin-like G protein-coupled receptor polypeptide encoded by any polynucleotide of claim1;
detecting binding of the test compound to the serotonin-like G protein-coupled receptor polypeptide, wherein a test compound which binds to the

polypeptide is identified as a potential therapeutic agent for decreasing the activity of a serotonin-like G protein-coupled receptor.

11. A method of screening for agents which regulate the activity of a serotonin-like G protein-coupled receptor, comprising the steps of:
contacting a test compound with a serotonin-like G protein-coupled receptor polypeptide encoded by any polynucleotide of claim 1; and
detecting a serotonin-like G protein-coupled receptor activity of the polypeptide, wherein a test compound which increases the serotonin-like G protein-coupled receptor activity is identified as a potential therapeutic agent for increasing the activity of the serotonin-like G protein-coupled receptor, and wherein a test compound which decreases the serotonin-like G protein-coupled receptor activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the serotonin-like G protein-coupled receptor.

12. A method of screening for agents which decrease the activity of a serotonin-like G protein-coupled receptor, comprising the steps of:
contacting a test compound with any polynucleotide of claim 1 and detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of serotonin-like G protein-coupled receptor.

13. A method of reducing the activity of serotonin-like G protein-coupled receptor, comprising the steps of:
contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any serotonin-like G protein-coupled receptor polypeptide of claim 4, whereby the activity of serotonin-like G protein-coupled receptor is reduced.

14. A reagent that modulates the activity of a serotonin-like G protein-coupled receptor polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claim 10 to 12.

15. A pharmaceutical composition, comprising:
the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.

16. Use of the expression vector of claim 2 or the reagent of claim 14 in the preparation of a medicament for modulating the activity of a serotonin-like G protein-coupled receptor in a disease.

17. Use of claim 16 wherein the disease is COPD, a cardiovascular disorder, cancer, a urinary disorder, obesity, diabetes, a CNS disorder, asthma, or a hematological disorder.

18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.

19. The cDNA of claim 18 which comprises SEQ ID NO:1.

20. The cDNA of claim 18 which consists of SEQ ID NO:1.

21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.

22. The expression vector of claim 21 wherein the polynucleotide consists of SEQ ID NO:1.

23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.

24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO:1.

25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO:2.

26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NO:2.

27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO:2.

28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising the steps of:
culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and
isolating the polypeptide.

29. The method of claim 28 wherein the expression vector comprises SEQ ID NO:1.

30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising the steps of:
hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO:1 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and
detecting the hybridization complex.

31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.

32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising:
a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO:1; and
instructions for the method of claim 30.

33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising the steps of:
contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and
detecting the reagent-polypeptide complex.

34. The method of claim 33 wherein the reagent is an antibody.

35. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2, comprising:
an antibody which specifically binds to the polypeptide; and
instructions for the method of claim 33.

36. A method of screening for agents which can modulate the activity of a human serotonin-like G protein-coupled receptor, comprising the steps of:
contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 27% identical to the amino acid sequence shown in SEQ ID NO:2 and (2) the amino acid sequence shown in SEQ ID NO:2; and

detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the human serotonin-like G protein-coupled receptor.

37. The method of claim 36 wherein the step of contacting is in a cell.

38. The method of claim 36 wherein the cell is *in vitro*.

39. The method of claim 36 wherein the step of contacting is in a cell-free system.

40. The method of claim 36 wherein the polypeptide comprises a detectable label.

41. The method of claim 36 wherein the test compound comprises a detectable label.

42. The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.

43. The method of claim 36 wherein the polypeptide is bound to a solid support.

44. The method of claim 36 wherein the test compound is bound to a solid support.

45. A method of screening for agents which modulate an activity of a human serotonin-like G protein-coupled receptor, comprising the steps of:
contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 27% identical to the amino acid sequence shown in SEQ ID NO:2 and (2) the amino acid sequence shown in SEQ ID NO:2; and

detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human serotonin-like G protein-coupled receptor, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human serotonin-like G protein-coupled receptor.

46. The method of claim 45 wherein the step of contacting is in a cell.

47. The method of claim 45 wherein the cell is *in vitro*.

48. The method of claim 45 wherein the step of contacting is in a cell-free system.

49. A method of screening for agents which modulate an activity of a human serotonin-like G protein-coupled receptor, comprising the steps of:
contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO:1; and
detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human serotonin-like G protein-coupled receptor.

50. The method of claim 49 wherein the product is a polypeptide.

51. The method of claim 49 wherein the product is RNA.

52. A method of reducing activity of a human serotonin-like G protein-coupled receptor, comprising the step of:

contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO:1, whereby the activity of a human serotonin-like G protein-coupled receptor is reduced.

53. The method of claim 52 wherein the product is a polypeptide.

54. The method of claim 53 wherein the reagent is an antibody.

55. The method of claim 52 wherein the product is RNA.

56. The method of claim 55 wherein the reagent is an antisense oligonucleotide.

57. The method of claim 56 wherein the reagent is a ribozyme.

58. The method of claim 52 wherein the cell is *in vitro*.

59. The method of claim 52 wherein the cell is *in vivo*.

60. A pharmaceutical composition, comprising:
a reagent which specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2; and
a pharmaceutically acceptable carrier.

61. The pharmaceutical composition of claim 60 wherein the reagent is an antibody.

62. A pharmaceutical composition, comprising:
a reagent which specifically binds to a product of a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO:1; and

a pharmaceutically acceptable carrier.

63. The pharmaceutical composition of claim 62 wherein the reagent is a ribozyme.

64. The pharmaceutical composition of claim 62 wherein the reagent is an antisense oligonucleotide.

65. The pharmaceutical composition of claim 62 wherein the reagent is an antibody.

66. A pharmaceutical composition, comprising:
an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2; and
a pharmaceutically acceptable carrier.

67. The pharmaceutical composition of claim 66 wherein the expression vector comprises SEQ ID NO:1.

68. A method of treating a serotonin-like G protein-coupled receptor dysfunction related disease, wherein the disease is selected from COPD, a cardiovascular disorder, cancer, a urinary disorder, obesity, diabetes, a CNS disorder, asthma, or a hematological disorder comprising the step of:
administering to a patient in need thereof a therapeutically effective dose of a reagent that modulates a function of a human serotonin-like G protein-coupled receptor, whereby symptoms of the serotonin-like G protein-coupled receptor disfunction related disease are ameliorated.

69. The method of claim 68 wherein the reagent is identified by the method of claim 36.

70. The method of claim 68 wherein the reagent is identified by the method of claim 45.

71. The method of claim 68 wherein the reagent is identified by the method of claim 49.

Fig. 1

atgggggatg agctggcacc ttgccctgtg ggcactacag cttggccggc cctgatccag
ctcatcagca agacacccctg catgccccaa gcagccagca acacttcctt gggcctgggg
gacctcaggg tgcccagctc catgctgtac tggcttttcc ttccctcaag cctgctggct
gcagccacac tggctgtcag ccccctgctg ctggtgacca tcctgcggaa ccaacggctg
cgacaggagc cccactacct gctcccggct aacatcctgc tctcagacct ggcctacatt
ctcctccaca tgctcatctc ctccagcagc ctgggtggct gggagctggg ccgcatggcc
tgtggcattc tcactgatgc tgtcttcgcc gcctgcacca gcaccatcct gtccttcacc
gccattgtgc tgcacaccta cctggcagtc atccatccac tgcgctacct ctccttcatg
tcccatgggg ctgcctggaa ggcagtggcc ctcatctggc tggtggcctg ctgcttcccc
acattcctta tttggctcag caagtggcag gatgcccagc tggaggagca aggagcttca
tacatcctac caccaagcat gggcacccag ccgggatgtg gcctcctggt cattgttacc
tacacctcca ttctgtgcgt tctgttcctc tgcacagctc tcattgccaa ctgtttctgg
aggatctatg cagaggccaa gacttcaggc atctgggggc agggctattc ccgggccagg
ggcaccctgc tgatccactc agtgctgatc acattgtacg tgagcacagg ggtggtgttc
tccctggaca tggtgctgac caggtaccac cacattgact ctgggactca cacatggctc
ctggcagcta acagtgaggt actcatgatg cttccccgtg ccatgctccc atacctgtac
ctgctccgct accggcagct gttgggcatg gtccggggcc acctcccatc caggaggcac
caggccatct ttaccatttc c**tag**

Fig. 2

MGDELAPCPV GTTAWPALIQ LISKTPCMPQ AASNTSLGLG DLRVPSSMLY WLFLPSSLLA
AATLAVSPLL LVTILRNQRL RQEPHYLLPA NILLSDLAYI LLHMLISSSS LGGWELGRMA
CGILTDAVFA ACTSTILSFT AIVLHTYLAV IHPLRYLSFM SHGAAWKAVA LIWLVACCFP
TFLIWLSKWQ DAQLEEQGAS YILPPSMGTQ PGCGLLVIVT YTSILCVLFL CTALIANCFW
RIYAEAKTSG IWGQGYSRAR GTLLIHSVLI TLYVSTGVVF SLDMVLTRYH HIDSGTHTWL
LAANSEVLMM LPRAMLPYLY LLRYRQLLGM VRGHLPSRRH QAIFTIS

Fig. 3

MEEPGAQCAP PPPAGSETWV PQANLSSAPS QNCSAKDYIY QDSISLPWKV LLVMLLALIT
LATTLSNAFV IATVYRTRKL HTPANYLIAS LAVTDLLVSI LVMPISTMYT VTGRWTLGQV
VCDFWLSSDI TCCTASILHL CVIALDRYWA ITDAVEYSAK RTPKRAAVMI ALVWVFSISI
SLPPFFWRQA KAEEEVSECV VNTDHILYTV YSTVGAFYFP TLLLIALYGR IYVEARSRIL
KQTPNRTGKR LTRAQLITDS PGSTSSVTSI NSRVPDVPSE SGSPVYVNQV KVRVSDALLE
KKKLMAARER KATKTLGIIL GAFIVCWLPF FIISLVMPIC KDACWFHLAI FDFFTWLGYL
NSLINPIIYT MSNEDFKQAF HKLIRFKCTS

Fig. 4

tgacaaagcc agccatccct gccaggaagc **atg**ggggatg agctggcacc ttgccctgtg
ggcactacag cttggccggc cctgatccag
ctcatcagca agacaccctg catgccccaa gcagccagca acacttcctt gggcctgggg
gacctcaggg tgcccagctc catgctgtac tggcttttcc ttccctcaag cctgctggct
gcagccacac tggctgtcag ccccctgctg ctggtgacca tcctgcggaa ccaacggctg
cgacaggagc cccactacct gctcccggct aacatcctgc tctcagacct ggcctacatt
ctcctccaca tgctcatctc ctccagcagc ctgggtggct gggagctggg ccgcatggcc
tgtggcattc tcactgatgc tgtcttcgcc gcctgcacca gcaccatcct gtccttcacc
gccattgtgc tgcacaccta cctggcagtc atccatccac tgcgctacct ctccttcatg
tcccatgggg ctgcctggaa ggcagtggcc ctcatctggc tggtggcctg ctgcttcccc
acattcctta tttggctcag caagtggcag gatgcccagc tggaggagca aggagcttca
tacatcctac caccaagcat gggcacccag ccgggatgtg gcctcctggt cattgttacc
tacacctcca ttctgtgcgt tctgttcctc tgcacagctc tcattgccaa ctgtttctgg
aggatctatg cagaggccaa gacttcaggc atctgggggc agggctattc ccgggccagg
ggcaccctgc tgatccactc agtgctgatc acattgtacg tgagcacagg ggtggtgttc
tccctggaca tggtgctgac caggtaccac cacattgact ctgggactca cacatggctc
ctggcagcta acagtgaggt actcatgatg cttccccgtg ccatgctccc atacctgtac
ctgctccgct accggcagct gttgggcatg gtccggggcc acctcccatc caggaggcac
caggccatct ttaccatttc c**tag**agttct tgagtccaca gtctggcaag ctga

Fig. 5

BLASTP - alignment of 414 against swiss|P28222|5H1B_HUMAN5-HYDROXYTRYPTAMINE 1B RECEPTOR (5-HT-1B) (SEROTONIN RECEPTOR)

(5-HT-1D-BETA)(S12).//:trembl|AB041370|AB041370_1 gene: "HTR1B";

Homo sapiens HTR1B gene for 5-hydroxytryptamine (serotonin) receptor 1B, complete cds.//:trembl|AB041377|AB041377_1 gene: "HTR1D";

Homo sapiens HTR1D gene for 5-hydroxytryptamine (serotonin) receptor 1D, complete cds. //:trembl|D10995|HSHGCR_1 gene: "HGCR1";

Human gene for serotonin 1B receptor,complete cds.//:trembl|M75128|HSHTR1E_1 gene: "HTR1D"; Human serotonin 1Db receptor (HTR1D) gene,complete cds. //:trembl|M83180|HSHTRA_1 Human serotonin receptor gene, complete cds. //:trembl|L09732|HSHTRD1A_1 Homo sapiens 5-hydroxytryptamine 1D receptor

gene, complete cds. //:trembl|AL049595|HSJ501M23_1 gene: "HTR1B";

Human DNA sequence from clone 501M23 on chromosome 6q13-14.3 Contains HTR1B (5-hydroxytryptamine (serotonin) receptor 1B gene), ESTs, CA repeat,STS and a CpG island //:trembl|M81590|HSSER1DRB_1 gene: "5-HT1D~"; Homo sapiens serotonin 1D receptor (5-HT1D~) mRNA, complete cds. //:trembl|AB041371|AB041371_1 gene: "HTR1B"; Pan troglodytes HTR1B gene for 5-hydroxytryptamine(serotonin) receptor 1B, complete cds. //:trembl|AB041378|AB041378_1 gene: "HTR1D"; Pan troglodytes HTR1D gene for

Fig. 5 (continued)

5-hydroxytryptamine (serotonin) receptor 1D, complete cds.
//:gp|AB041370|7592929 gene: "HTR1B"; Homo sapiens HTR1B gene for 5-hydroxytryptamine (serotonin) receptor 1B, complete cds.
//:gp|AB041371|7592931 gene: "HTR1B"; Pan troglodytes HTR1B gene for 5-hydroxytryptamine (serotonin)receptor 1B, complete cds. //:gp|AB041377|7592944 gene:"HTR1D"; Homo sapiens HTR1D gene for 5-hydroxytryptamine (serotonin) receptor 1D, complete cds. //:gp|AB041378|7592946 gene: "HTR1D"; Pan troglodytes HTR1D gene for 5-hydroxytryptamine (serotonin)receptor 1D, complete cds.
//:gp|AL049595|5629918 gene: "HTR1B"; Human DNA sequence from clone 501M23 on chromosome 6q13-14.3 Contains HTR1B(5-hydroxytryptamine (serotonin) receptor 1B gene), ESTs, CA repeat, STS and a CpG island, complete sequence.//:gp|D10995|219679 gene: "HGCR1"; Human gene for serotonin 1B receptor,complete cds. //:gp|M75128|184460 gene: "HTR1D"; Human serotonin 1Db receptor (HTR1D) gene, complete cds. //:gp|M83180|184464 product: "serotonin receptor"; Human serotonin receptor gene, complete cds. //:gp|L09732|184468 product: "5-hyroxytryptamine 1D receptor"; Homo sapiens 5-hydroxytryptamine 1D receptor gene, complete cds.//:gp|M81590|338026 gene: "5-HT1D~"; Homo sapiens serotonin 1D receptor (5-HT1D~) mRNA, complete cds.

This hit is scoring at : 5e-12 (expectation value)
Alignment length (overlap) : 272
Identities : 26 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb_1_;

Fig. 5 (continued)

```
Q:       7 PCPVGTTAWPALIQLISKTPCMPQAASNTSLGLGDLRVPSSMLYWLFLPSSLLAAATLAV
           P P.G:..W . ..L S       A.S... . . :  .S .L W .L  .LLA..TLA.
H:      11 PPPAGSETWVPQANLSS-------APSQNCSAKDYIYQDSISLPWKVLLVMLLALITLAT

           S---PLLLVTILRNQRLRQEPHYLLPANILLSDLAYILLHMLISS--SSLGGWELGRMAC
           :   ..:::.T:.R.::L....:YL: A:.:.::DL.. :L M IS:  : .G W.LG::.C
           TLSNAFVIATVYRTRKLHTPANYLI-ASLAVTDLLVSILVMPISTMYTVTGRWTLGQVVC

           GILTDAVFAACTSTILSFTAIVLHTYLAVIHPLRYLSFMSHGAAWKAVALIWL--VACCF
           .. ..: ...CT::IL....I.L..Y A:...:.Y : .:  .A  .:AL:W:  ::...
           DFWLSSDITCCTASILHLCVIALDRYWAITDAVEYSAKRTPKRAAVMIALVWVFSISISL

           PTFLIWLSKWQDAQLEEQGASYILPPSMGTQPGCGLLVIVTYTSILCVLFLCTALIANCF
           P.F.     W:.A:.EE: :. :: ..          ::.T. S.: ..:. T.L:. .:
           PPFF-----WRQAKAEEEVSECVVNTDH---------ILYTVYSTVGAFYFPTLLLIALY

           WRIYAEAKTSGIWGQGYSRARGTLLIHSVLIT     271
            RIY.EA: S I  Q  :R. G. L..: LIT
           GRIYVEAR-SRILKQTPNRT-GKRLTRAQLIT     258
```

Fig. 6

HMMPFAM - alignment of 414 against pfam|hmm|7tm_1 7 transmembrane receptor (rhodopsin family)

This hit is scoring at : 31.6
Scoring matrix : BLOSUM62 (used to infer consensus pattern)

```
Q:      69 LLLVTILRNQRLRQEPHYLLpANILLSDLAYILLHMLISSSSLGG----WELGRMACGIL
           L::::.ILR.::LR...:..: .N:.::DL.::L.    :   L G    W..G...C ::
H:       4 LVilvilrtkklrtptnifi.lNLAvADLLflltlppwalyylvggsedWpfGsalCklv

          TDAVFAACTSTILSFTAIVLHTYLAVIHPLRYLSFMS-HGAAWKAVALIWLVACCFPTFL
          T . .. . ::IL .TAI :..YLA::HPLRY . .:    .A  .:.L:W::A.....
          taldvvnmyaSillLtaISiDRYlAIvhPlryrrrrtsprrAkvvillvWvlalllslPp

          IWLSKWQDAQLEEQ---GASYILPPSMGTQPGCG---LLVIVTYTSILCVLFLCTALIAN
          : .S  :..:  .        :.  .. .:. ..    L  ....S.L. ..L ..:I.
          llfswvktveegngtlnvnvtvClidfpeestasvstwlrsyvllstlvgFllPllvilv

          CFWRIYAEAKtsgiwgqgysrargtllihSVLITLYVSTGVVF     280
          C: RI....:                    ... TL.V . V..
          cYtrIlrtlr.....................kaaktllvvvvvFv     206
```

Fig. 7

GENSCAN 1.0 Date run: 24-Jan-101 Time: 09:08:50

Sequence AC060810 : 159288 bp : 44.22% C+G : Isochore 2 (43 - 51 C+G%)

Parameter matrix: HumanIso.smat

Predicted genes/exons: Gn.Ex Type S .Begin ...End .Len Fr Ph I/Ac Do/T CodRg P.... Tscr..

4.00	Prom	+	71395	71434	40							-6.66
4.01	Sngl	+	78075	79118	1044	2	0	94	40	1004	0.994	91.35
4.02	PlyA	+	79642	79647	6							1.05

Fig. 8

>AC060810|GENSCAN_predicted_peptide_4|347_aa
MGDELAPCPVGTTAWPALIQLISKTPCMPQAASNTSLGLGDLRVPSSMLYWLFLPSSLLA
AATLAVSPLLLVTILRNQRLRQEPHYLLPANILLSDLAYILLHMLISSSSLGGWELGRMA
CGILTDAVFAACTSTILSFTAIVLHTYLAVIHPLRYLSFMSHGAAWKAVALIWLVACCFP
TFLIWLSKWQDAQLEEQGASYILPPSMGTQPGCGLLVIVTYTSILCVLFLCTALIANCFW
RIYAEAKTSGIWGQGYSRARGTLLIHSVLITLYVSTGVVFSLDMVLTRYHHIDSGTHTWL
LAANSEVLMMLPRAMLPYLYLLRYRQLLGMVRGHLPSRRHQAIFTIS

>AC060810|GENSCAN_predicted_CDS_4|1044_bp
atggggatgagctggcaccttgccctgtgggcactacagcttggccggccctgatccag
ctcatcagcaagacaccctgcatgccccaagcagccagcaacacttccttgggcctgggg
gacctcagggtgcccagctccatgctgtactggcttttccttccctcaagcctgctggct
gcagccacactggctgtcagccccctgctgctggtgaccatcctgcggaaccaacggctg
cgacaggagccccactacctgctcccggctaacatcctgctctcagacctggcctacatt
ctcctccacatgctcatctcctccagcagcctgggtggctgggagctgggccgcatggcc
tgtggcattctcactgatgctgtcttcgccgcctgcaccagcaccatcctgtccttcacc
gccattgtgctgcacacctacctggcagtcatccatccactgcgctacctctccttcatg
tcccatggggctgcctggaaggcagtggccctcatctggctggtggcctgctgcttcccc
acattccttatttggctcagcaagtggcaggatgcccagctggaggagcaaggagcttca
tacatcctaccaccaagcatgggcacccagccgggatgtggcctcctggtcattgttacc
tacacctccattctgtgcgttctgttcctctgcacagctctcattgccaactgtttctgg
aggatctatgcagaggccaagacttcaggcatctgggggcagggctattcccgggccagg
ggcaccctgctgatccactcagtgctgatcacattgtacgtgagcacaggggtggtgttc
tccctggacatggtgctgaccaggtaccaccacattgactctgggactcacacatggctc
ctggcagctaacagtgaggtactcatgatgcttccccgtgccatgctcccatacctgtac

Fig. 8 (continued)

ctgctccgctaccggcagctgttgggcatggtccggggccacctcccatccaggaggcac
caggccatctttaccatttcctag

A BLAST search against the nrdb_SNP database identified single nucleotide

Polymorphisms for #414. SNP sequence, SNP:1275729_867778, cover the range from 924 to 954 bp, which is located on transmembrane helix 7.
This is a nonconservative SNP in which Proline is changed to Threonine.

Fig. 9

BLASTN - alignment of 414_DNA against SNP:1275729_867778SNP:OVLP-000621-121708 NR:2 >SNP:1942595_867780 SNP:OVLP-000804-412068 NR:2

>SNP:384138_867782 SNP:OVLP-000925-720069 NR:2

This hit is scoring at : 7e-18 (expectation value)
Alignment length (overlap) : 51
Identities : 98 %
Scoring matrix : blastn (used to infer consensus pattern)
Query reading frame : +1
Hit reading frame : -1
Database searched : dbSNP_nrdb.fasta

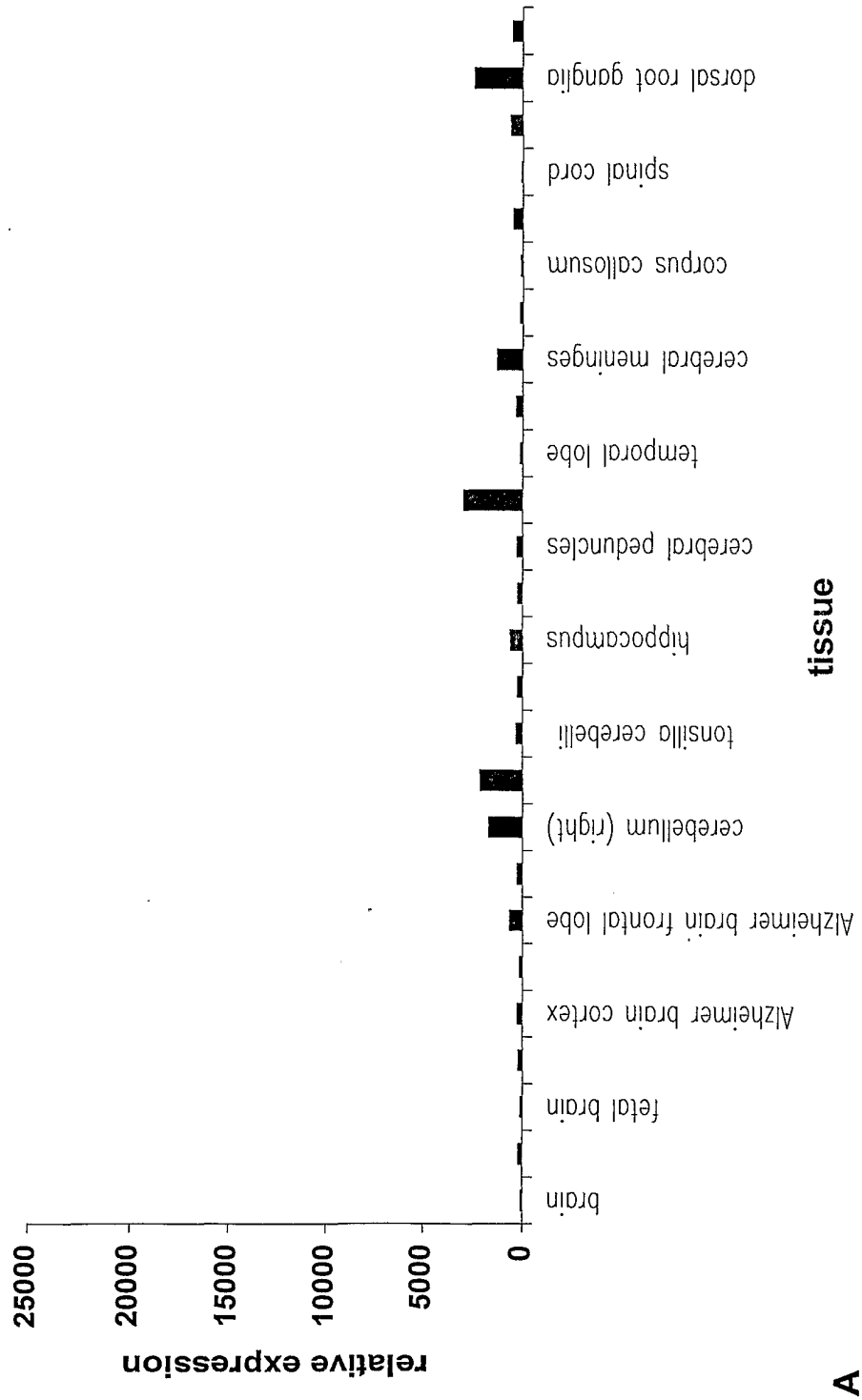
```
Q:    924 catgatgcttccccgtgccatgctcccatacctgtacctgctccgctaccg     974
          CATGATGCTTCCCCGTGCCATGCTC CATACCTGTACCTGCTCCGCTACCG
H:     51 catgatgcttccccgtgccatgctcacatacctgtacctgctccgctaccg
```

Fig. 10

MGDELAPCPV GTTAWPALIQ LISKTPCMPQ AASNTSLGLG DLRVPSSMLY WLFLPSSLLA AATLAVSPLL LVTILRNQRL RQEPHYLLPA NILLSDLAYI LLHMLISSSS LGGWELGRMA

CGILTDAVFA ACTSTILSFT AIVLHTYLAV IHPLRYLSFM SHGAAWKAVA LIWLVACCFP TFLIWLSKWQ DAQLEEQGAS YILPPSMGTQ PGCGLLVIVT YTSILCVLFL CTALIANCFW

RIYAEAKTSG IWGQGYSRAR GTLLIHSVLI TLYVSTGVVF SLDMVLTRYH HIDSGTHTWL LAANSEVLMM LPRAMLPYLY LLRYRQLLGM VRGHLPSRRH QAIFTIS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25000
20000
15000
10000
5000
0
brain
fetal brain
Alzheimer brain cortex
Alzheimer brain frontal lobe
cerebellum (right)
tonsilla cerebelli
hippocampus
cerebral peduncles
temporal lobe
cerebral meninges
corpus callosum
spinal cord
dorsal root ganglia
tissue
A

Fig. 11

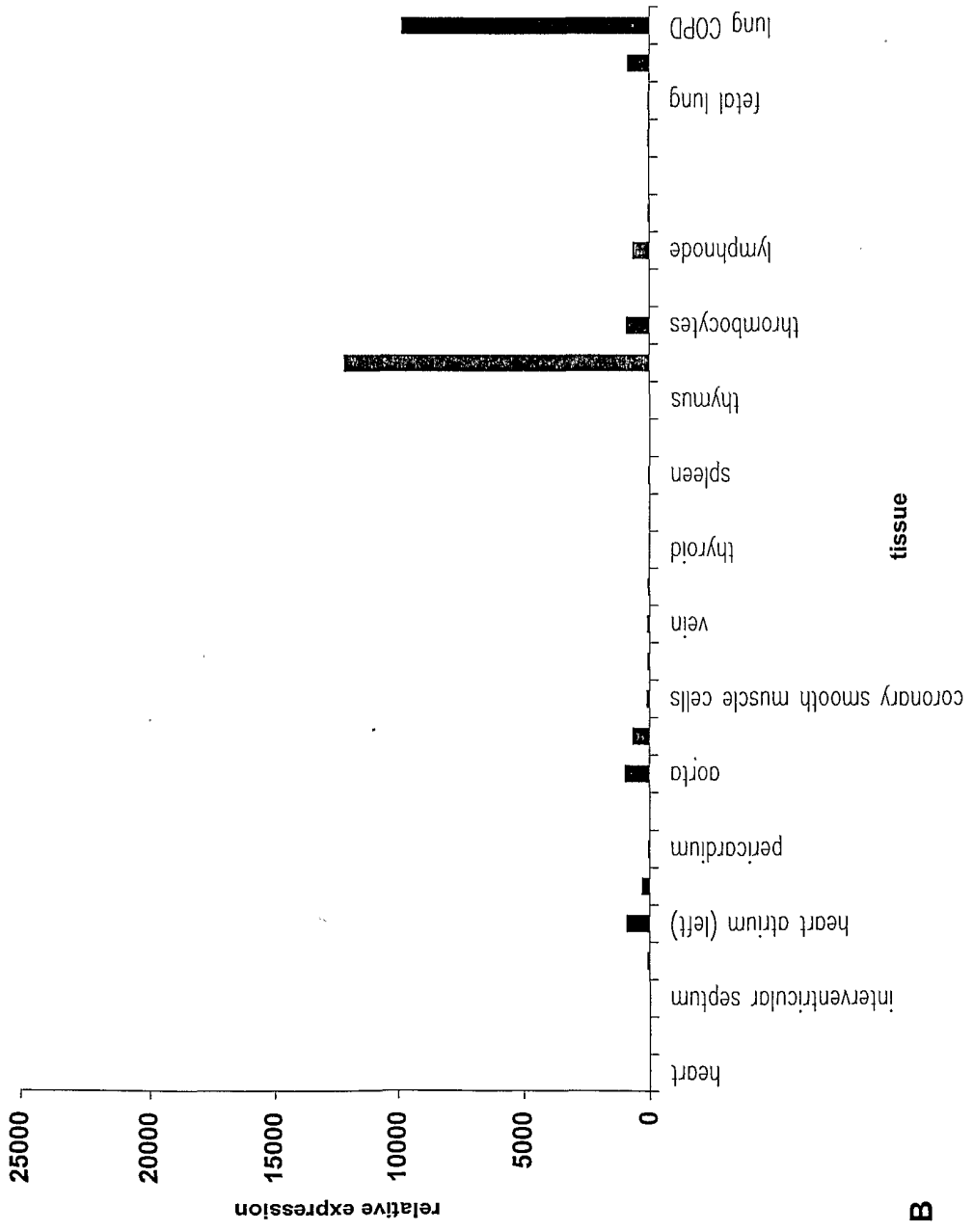
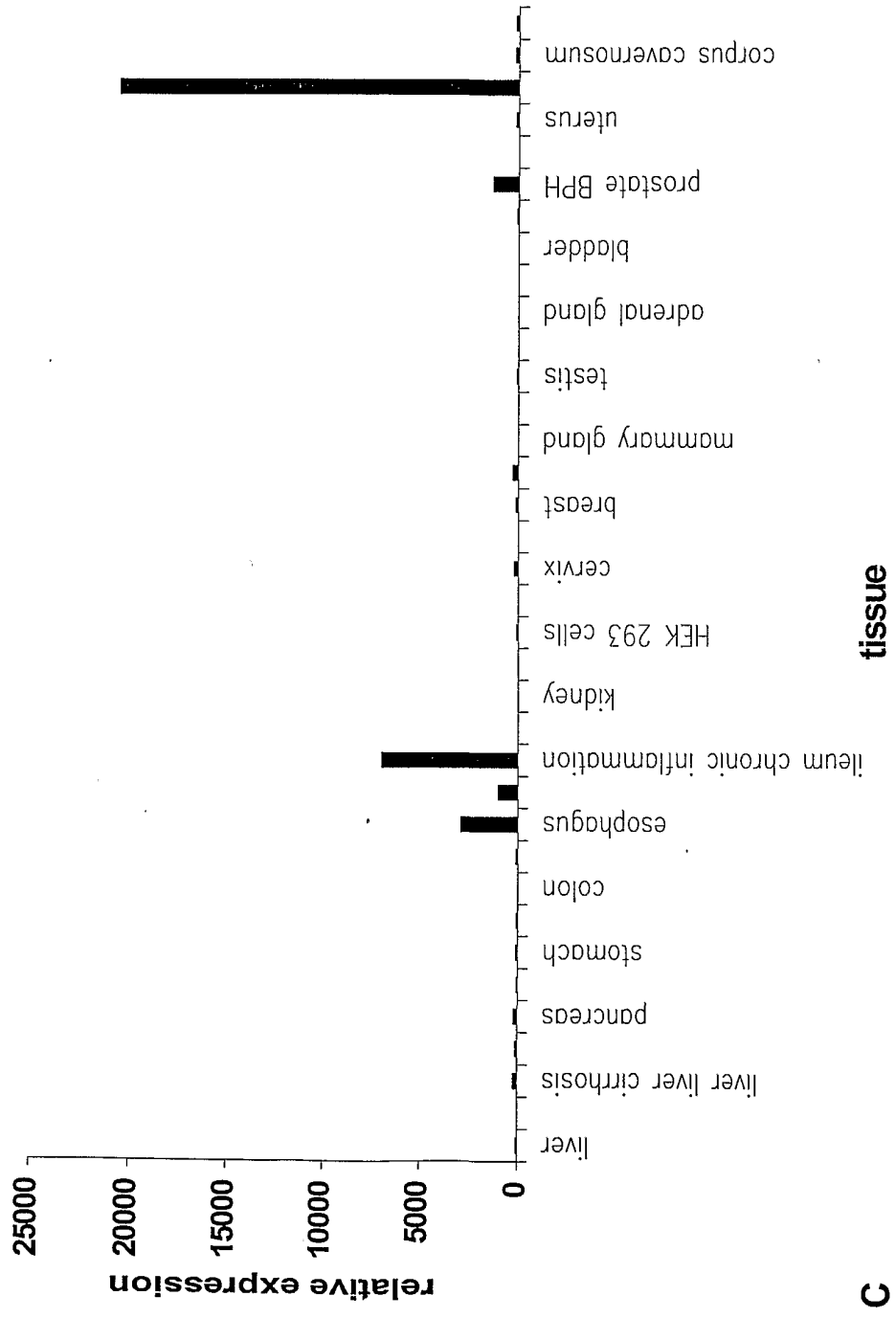

B
relative expression
25000
20000
15000
10000
5000
0
tissue
heart
interventricular septum
heart atrium (left)
pericardium
aorta
coronary smooth muscle cells
vein
thyroid
spleen
thymus
thrombocytes
lymphnode
fetal lung
lung COPD

Fig. 12


C
relative expression
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20000
15000
10000
5000
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liver
liver liver cirrhosis
pancreas
stomach
colon
esophagus
ileum chronic inflammation
kidney
HEK 293 cells
cervix
breast
mammary gland
testis
adrenal gland
bladder
prostate BPH
uterus
corpus cavernosum
tissue

Fig. 13

SEQUENCE LISTING

<110> Bayer AG

<120> REGULATION OF HUMAN SEROTONIN-LIKE G PROTEIN-COUPLED RECEPTOR

<130> LIO290 Foreign Countries

<150> US 60/264,071

<151> 2002-01-26

<150> US 60/324,054

<151> 2001-09-24

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Ser Asn Thr Ser Leu Gly Leu Gly Asp Leu Arg Val Pro Ser Ser Met
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Leu Tyr Trp Leu Phe Leu Pro Ser Ser Leu Leu Ala Ala Ala Thr Leu
    50                  55                  60

Ala Val Ser Pro Leu Leu Leu Val Thr Ile Leu Arg Asn Gln Arg Leu
65                  70                  75                  80

Arg Gln Glu Pro His Tyr Leu Leu Pro Ala Asn Ile Leu Leu Ser Asp
                85                  90                  95

Leu Ala Tyr Ile Leu Leu His Met Leu Ile Ser Ser Ser Ser Leu Gly
            100                 105                 110

Gly Trp Glu Leu Gly Arg Met Ala Cys Gly Ile Leu Thr Asp Ala Val
        115                 120                 125

Phe Ala Ala Cys Thr Ser Thr Ile Leu Ser Phe Thr Ala Ile Val Leu
    130                 135                 140

His Thr Tyr Leu Ala Val Ile His Pro Leu Arg Tyr Leu Ser Phe Met
145                 150                 155                 160

Ser His Gly Ala Ala Trp Lys Ala Val Ala Leu Ile Trp Leu Val Ala
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Cys Cys Phe Pro Thr Phe Leu Ile Trp Leu Ser Lys Trp Gln Asp Ala
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Thr Gln Pro Gly Cys Gly Leu Leu Val Ile Val Thr Tyr Thr Ser Ile
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Leu Cys Val Leu Phe Leu Cys Thr Ala Leu Ile Ala Asn Cys Phe Trp
225                 230                 235                 240

Arg Ile Tyr Ala Glu Ala Lys Thr Ser Gly Ile Trp Gly Gln Gly Tyr
                245                 250                 255

Ser Arg Ala Arg Gly Thr Leu Leu Ile His Ser Val Leu Ile Thr Leu
            260                 265                 270

Tyr Val Ser Thr Gly Val Val Phe Ser Leu Asp Met Val Leu Thr Arg
        275                 280                 285

Tyr His His Ile Asp Ser Gly Thr His Thr Trp Leu Leu Ala Ala Asn
    290                 295                 300

Ser Glu Val Leu Met Met Leu Pro Arg Ala Met Leu Pro Tyr Leu Tyr
305                 310                 315                 320

Leu Leu Arg Tyr Arg Gln Leu Leu Gly Met Val Arg Gly His Leu Pro
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Cys Ser Ala Lys Asp Tyr Ile Tyr Gln Asp Ser Ile Ser Leu Pro Trp
        35                  40                  45

Lys Val Leu Leu Val Met Leu Leu Ala Leu Ile Thr Leu Ala Thr Thr
    50                  55                  60

Leu Ser Asn Ala Phe Val Ile Ala Thr Val Tyr Arg Thr Arg Lys Leu
65                  70                  75                  80

His Thr Pro Ala Asn Tyr Leu Ile Ala Ser Leu Ala Val Thr Asp Leu
                85                  90                  95

Leu Val Ser Ile Leu Val Met Pro Ile Ser Thr Met Tyr Thr Val Thr
            100                 105                 110

Gly Arg Trp Thr Leu Gly Gln Val Val Cys Asp Phe Trp Leu Ser Ser
        115                 120                 125

Asp Ile Thr Cys Cys Thr Ala Ser Ile Leu His Leu Cys Val Ile Ala
    130                 135                 140

Leu Asp Arg Tyr Trp Ala Ile Thr Asp Ala Val Glu Tyr Ser Ala Lys
145                 150                 155                 160
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Arg Thr Pro Lys Arg Ala Ala Val Met Ile Ala Leu Val Trp Val Phe
                165                 170                 175

Ser Ile Ser Ile Ser Leu Pro Pro Phe Phe Trp Arg Gln Ala Lys Ala
            180                 185                 190

Glu Glu Glu Val Ser Glu Cys Val Val Asn Thr Asp His Ile Leu Tyr
        195                 200                 205

Thr Val Tyr Ser Thr Val Gly Ala Phe Tyr Phe Pro Thr Leu Leu Leu
    210                 215                 220

Ile Ala Leu Tyr Gly Arg Ile Tyr Val Glu Ala Arg Ser Arg Ile Leu
225                 230                 235                 240

Lys Gln Thr Pro Asn Arg Thr Gly Lys Arg Leu Thr Arg Ala Gln Leu
                245                 250                 255

Ile Thr Asp Ser Pro Gly Ser Thr Ser Ser Val Thr Ser Ile Asn Ser
            260                 265                 270

Arg Val Pro Asp Val Pro Ser Glu Ser Gly Ser Pro Val Tyr Val Asn
        275                 280                 285

Gln Val Lys Val Arg Val Ser Asp Ala Leu Leu Glu Lys Lys Lys Leu
    290                 295                 300

Met Ala Ala Arg Glu Arg Lys Ala Thr Lys Thr Leu Gly Ile Ile Leu
305                 310                 315                 320

Gly Ala Phe Ile Val Cys Trp Leu Pro Phe Phe Ile Ile Ser Leu Val
                325                 330                 335

Met Pro Ile Cys Lys Asp Ala Cys Trp Phe His Leu Ala Ile Phe Asp
            340                 345                 350
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Phe Phe Thr Trp Leu Gly Tyr Leu Asn Ser Leu Ile Asn Pro Ile Ile
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Arg Phe Lys Cys Thr Ser
385 390

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gcagccagca acacttcctt gggcctgggg gacctcaggg tgcccagctc catgctgtac 180

tggcttttcc ttccctcaag cctgctggct gcagccacac tggctgtcag ccccctgctg 240

ctggtgacca tcctgcggaa ccaacggctg cgacaggagc cccactacct gctcccggct 300

aacatcctgc tctcagacct ggcctacatt ctcctccaca tgctcatctc ctccagcagc 360

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