



(72) BONNERT, TIMOTHY PETER, GB

(72) WHITING, PAUL JOHN, GB

(71) MERCK SHARP & DOHME LIMITED, GB

(51) Int.Cl.⁶ C12N 15/12, C07K 14/705, C12Q 1/68, G01N 33/68,
G01N 33/50, A61K 38/17, C12N 5/10

(30) 1997/04/25 (9708479.2) GB

(54) **SOUS-UNITE THETA HUMAINE DU RECEPTEUR DE GABA-A**

(54) **HUMAN THETA SUBUNIT OF THE GABA-A RECEPTOR**

(57) La présente invention concerne le clonage d'une nouvelle séquence d'ADN complémentaire codant la sous-unité de récepteur thêta du récepteur de GABA_A; l'invention concerne également des lignées cellulaires eucaryotes co-transfectées de manière stable, capables d'exprimer un récepteur de GABA_A, ledit récepteur comprenant la nouvelle sous-unité de récepteur thêta; l'invention concerne enfin l'utilisation de ces lignées cellulaires dans le criblage et la conception de médicaments ayant un effet sur le récepteur de GABA_A.

(57) The present invention relates to the cloning of a novel cDNA sequence encoding the theta receptor subunit of the GABA_A receptor; to stably co-transfected eukaryotic cell lines capable of expressing a GABA_A receptor, which receptor comprises the novel theta receptor subunit; and to the use of such cell lines in screening for and designing medicaments which act upon the GABA_A receptor.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification⁶ : C12N 15/12, C07K 14/705, C12N 5/10, A61K 38/17, G01N 33/50, C12Q 1/68, G01N 33/68</p>	A1	<p>(11) International Publication Number: WO 98/49293</p> <p>(43) International Publication Date: 5 November 1998 (05.11.98)</p>
<p>(21) International Application Number: PCT/GB98/01206</p> <p>(22) International Filing Date: 24 April 1998 (24.04.98)</p> <p>(30) Priority Data: 9708479.2 25 April 1997 (25.04.97) GB</p> <p>(71) Applicant (for all designated States except US): MERCK SHARP & DOHME LIMITED [GB/GB]; Hertford Road, Hoddesdon, Hertfordshire EN11 9BU (GB).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): BONNERT, Timothy, Peter [GB/GB]; Terlings Park, Eastwick road, Harlow, Essex CM20 2QR (GB). WHITING, Paul, John [GB/GB]; Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR (GB).</p> <p>(74) Agent: HISCOCK, Ian; Merck & Co., Inc, European Patent Dept., Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR (GB).</p>	<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
<p>(54) Title: HUMAN THETA SUBUNIT OF THE GABA-A RECEPTOR</p>		
<p>(57) Abstract</p> <p>The present invention relates to the cloning of a novel cDNA sequence encoding the theta receptor subunit of the GABA_A receptor; to stably co-transfected eukaryotic cell lines capable of expressing a GABA_A receptor, which receptor comprises the novel theta receptor subunit; and to the use of such cell lines in screening for and designing medicaments which act upon the GABA_A receptor.</p>		

HUMAN THETA SUBUNIT OF THE GABA-A RECEPTOR

This invention concerns the cloning of a novel cDNA sequence encoding a particular subunit of the human GABA_A receptor. In addition, 5 the invention relates to a stable cell line capable of expressing said cDNA and to the use of the cell line in a screening technique for the design and development of subtype-specific medicaments.

Gamma-amino butyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system. It mediates fast synaptic 10 inhibition by opening the chloride channel intrinsic to the GABA_A receptor. This receptor comprises a multimeric protein of molecular size 230-270 kDa with specific binding sites for a variety of drugs including benzodiazepines, barbiturates and β -carbolines, in addition to sites for the agonist ligand GABA (for reviews see MacDonald and Olsen, *Ann. Rev.* 15 *Neurosci.*, 1994, 17, 569; and Whiting *et al.*, *Int. Rev. Neurobiol.*, 1995, 38, 95).

Molecular biological studies demonstrate that the receptor is composed of several distinct types of subunit, which are divided into four classes (α , β , γ and δ) based on their sequence similarities. To date, in 20 mammals, six types of α (Schofield *et al.*, *Nature (London)*, 1987, 328, 221; Levitan *et al.*, *Nature (London)*, 1988, 335, 76; Ymer *et al.*, *EMBO J.*, 1989, 8, 1665; Pritchett & Seeberg, *J. Neurochem.*, 1990, 54, 802; Luddens *et al.*, *Nature (London)*, 1990, 346, 648; and Khrestchatisky *et al.*, *Neuron*, 1989, 3, 745), three types of β (Ymer *et al.*, *EMBO J.*, 1989, 8, 1665), three 25 types of γ (Ymer *et al.*, *EMBO J.*, 1990, 9, 3261; Shivers *et al.*, *Neuron*, 1989, 3, 327; and Knoflach *et al.*, *FEBS Lett.*, 1991, 293, 191) and one δ subunit (Shivers *et al.*, *Neuron*, 1989, 3, 327) have been identified. More recently, a further member of the GABA receptor gene family, ϵ , has been identified (Davies *et al.*, *Nature*, 1997, 385, 820). The polypeptide is 506 30 amino acids in length and exhibits greatest amino acid sequence identity

with the GABA_A receptor γ_3 subunit (47%), although this degree of homology is not sufficient for it to be classified as a fourth γ subunit.

The differential distribution of many of the subunits has been characterised by *in situ* hybridisation (Shivers *et al.*, *Neuron*, 1989, **3**, 327; Wisden *et al.*, *J. Neurosci.*, 1992, **12**, 1040; and Laurie *et al.*, *J. Neurosci.*, 1992, **12**, 1063) and this has permitted it to be speculated which subunits, by their co-localisation, could theoretically exist in the same receptor complex.

Various combinations of subunits have been co-transfected into cells to identify synthetic combinations of subunits whose pharmacology parallels that of *bona fide* GABA_A receptors *in vivo* (Pritchett *et al.*, *Science*, 1989, **245**, 1389; Pritchett and Seeberg, *J. Neurochem.*, 1990, **54**, 1802; Luddens *et al.*, *Nature (London)*, 1990, **346**, 648; Hadingham *et al.*, *Mol. Pharmacol.*, 1993, **43**, 970; and Hadingham *et al.*, *Mol. Pharmacol.*, 1993, **44**, 1211). This approach has revealed that, in addition to an α and β subunit, either γ_1 or γ_2 (Pritchett *et al.*, *Nature (London)*, 1989, **338**, 582; Ymer *et al.*, *EMBO J.*, 1990, **9**, 3261; and Wafford *et al.*, *Mol. Pharmacol.*, 1993, **44**, 437) or γ_3 (Herb *et al.*, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 1433; Knoflach *et al.*, *FEBS Lett.*, 1991, **293**, 191; and Wilson-Shaw *et al.*, *FEBS Lett.*, 1991, **284**, 211) is also generally required to confer benzodiazepine sensitivity, and that the benzodiazepine pharmacology of the expressed receptor is largely dependent on the identity of the α and γ subunits present. Receptors containing a δ subunit (i.e. $\alpha\beta\delta$) do not appear to bind benzodiazepines (Shivers *et al.*, *Neuron*, 1989, **3**, 327; and Quirk *et al.*, *J. Biol. Chem.*, 1994, **269**, 16020). Combinations of subunits have been identified which exhibit the pharmacological profile of a BZ₁ type receptor ($\alpha_1\beta_1\gamma_2$) and a BZ₂ type receptor ($\alpha_2\beta_1\gamma_2$ or $\alpha_3\beta_1\gamma_2$, Pritchett *et al.*, *Nature (London)*, 1989, **338**, 582), as well as GABA_A receptors with a novel pharmacology, $\alpha_5\beta_2\gamma_2$ (Pritchett and Seeberg, *J. Neurochem.*, 1990, **54**, 1802), $\alpha_4\beta_2\gamma_2$ (Wisden *et al.*, *FEBS Lett.*, 1991, **289**, 227) and $\alpha_6\beta_2\gamma_2$

(Luddens *et al.*, *Nature (London)*, 1990, 346, 648). The pharmacology of these expressed receptors appears similar to that of those identified in brain tissue by radioligand binding, and biochemical experiments have begun to determine the subunit composition of native GABA receptors
5 (McKernan & Whiting, *Tr. Neurosci.*, 1996, 19, 139). The exact structure of receptors *in vivo* has yet to be definitively elucidated.

The present invention relates to a new class of GABA receptor subunit, hereinafter referred to as the theta subunit (θ subunit).

The nucleotide sequence for the theta subunit, together with its
10 deduced amino acid sequence corresponding thereto, is depicted in Figure 1 of the accompanying drawings.

The present invention accordingly provides, in a first aspect, a DNA molecule encoding the theta subunit of the human GABA receptor comprising all or a portion of the sequence depicted in Figure 1, or a
15 modified human sequence.

In an alternative aspect, the present invention provides a DNA molecule encoding the theta subunit of the human GABA receptor comprising all or a portion of the sequence depicted in Figure 2, or a modified human sequence.

20 The term "modified human sequence" as used herein refers to a variant of the DNA sequences depicted in Figure 1 and Figure 2. Such variants may be naturally occurring allelic variants or non-naturally occurring or "engineered" variants. Allelic variation is well known in the art in which the nucleotide sequence may have a substitution, deletion or
25 addition of one or more nucleotides without substantial alteration of the function of the encoded polypeptide. Particularly preferred allelic variants arise from nucleotide substitution based on the degeneracy of the genetic code.

The sequencing of the novel cDNA molecules in accordance with the
30 invention can conveniently be carried out by the standard procedure described in accompanying Example 1; or may be accomplished by

alternative molecular cloning techniques which are well known in the art, such as those described by Maniatis *et al.* in *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, New York, 2nd edition, 1989.

5 In a further aspect, the present invention also relates to polynucleotides (for example, cDNA, genomic DNA or synthetic DNA) which hybridize under stringent conditions to the DNA molecules depicted in Figure 1 and Figure 2. As used herein, the term "stringent conditions" will be understood to require at least 95% and preferably at least 97%
10 identity between the hybridized sequences. Polynucleotides which hybridize under stringent conditions to the DNA molecules depicted in Figure 1 and Figure 2 preferably encode polypeptides which exhibit substantially the same biological activity or function as the polypeptides depicted in Figure 1 and Figure 2, respectively.

15 The present invention further relates to a GABA theta subunit polypeptide which has the deduced amino acid sequence of Figure 1 or Figure 2, as well as fragments, analogs and derivatives thereof.

 The terms "fragment", "derivative" and "analog" when referring to the polypeptide of Figure 1 or Figure 2, means a polypeptide which retains
20 essentially the same biological activity or function as the polypeptide depicted in Figure 1 or Figure 2. Thus, an analog may be, for example, a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

 The polypeptide of the present invention may be a recombinant
25 polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

 The fragment, derivative or analog of the polypeptide of Figure 1 or Figure 2 may be one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue
30 (preferably a conserved amino acid residue) and such substituted amino acid residues may or may not be one encoded by the genetic code; or one in

which one or more of the amino acid residues includes a substituent group; or one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); or one in which the additional amino acids
5 are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the technical capabilities of those skilled in the art.

10 The polypeptides and DNA molecules of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally
15 occurring). For example, a naturally-occurring DNA molecule or polypeptide present in a living animal is not isolated, but the same DNA molecule or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such DNA molecules could be part of a vector and/or such DNA molecules or polypeptides could be part
20 of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

In another aspect, the invention provides a recombinant expression vector comprising the nucleotide sequence of the human GABA receptor theta subunit together with additional sequences capable of directing the
25 synthesis of the said human GABA receptor theta subunit in cultures of stably co-transfected eukaryotic cells.

The term "expression vectors" as used herein refers to DNA sequences that are required for the transcription of cloned copies of recombinant DNA sequences or genes and the translation of their mRNAs
30 in an appropriate host. Such vectors can be used to express eukaryotic genes in a variety of hosts such as bacteria, blue-green algae, yeast cells,

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

The term "cloning vector" as used herein refers to a DNA molecule, usually a small plasmid or bacteriophage DNA capable of self-replication in a host organism, and used to introduce a fragment of foreign DNA into
15 a host cell. The foreign DNA combined with the vector DNA constitutes a recombinant DNA molecule which is derived from recombinant technology. Cloning vectors may include plasmids, bacteriophages, viruses and cosmids.

The recombinant expression vector in accordance with the invention
20 may be prepared by inserting the nucleotide sequence of the GABA theta subunit into a suitable precursor expression vector (hereinafter referred to as the "precursor vector") using conventional recombinant DNA methodology known from the art. The precursor vector may be obtained commercially, or constructed by standard techniques from known
25 expression vectors. The precursor vector suitably contains a selection marker, typically an antibiotic resistance gene, such as the neomycin or ampicillin resistance gene. The precursor vector preferably contains a neomycin resistance gene, adjacent the SV40 early splicing and polyadenylation region; an ampicillin resistance gene; and an origin of
30 replication, e.g. pBR322 ori. The vector also preferably contains an inducible promoter, such as MMTV-LTR (inducible with dexamethasone)

or metallothionin (inducible with zinc), so that transcription can be controlled in the cell line of this invention. This reduces or avoids any problem of toxicity in the cells because of the chloride channel intrinsic to the GABA_A receptor.

5 One suitable precursor vector is pMAMneo, available from Clontech Laboratories Inc. (Lee *et al.*, *Nature*, 1981, **294**, 228; and Sardet *et al.*, *Cell*, 1989, **56**, 271). Alternatively the precursor vector pMSGneo can be constructed from the vectors pMSG and pSV2neo.

10 The recombinant expression vector of the present invention is then produced by cloning the GABA receptor theta subunit cDNA into the above precursor vector. The receptor subunit cDNA is subcloned from the vector in which it is harboured, and ligated into a restriction enzyme site, e.g. the Hind III site, in the polylinker of the precursor vector, for example pMAMneo or pMSGneo, by standard cloning methodology known from the art, and in particular by techniques analogous to those described herein. 15 Before this subcloning, it is often advantageous, in order to improve expression, to modify the end of the theta subunit cDNA with additional 5' untranslated sequences, for example by modifying the 5' end of the theta subunit DNA by addition of 5' untranslated region sequences from the α_1 subunit DNA. 20 Alternatively, expression of the theta subunit cDNA may be modified by the insertion of an epitope tag sequence such as c-myc.

According to a further aspect of the present invention, there is provided a stably co-transfected eukaryotic cell line capable of expressing a GABA receptor, which receptor comprises the theta receptor subunit, at 25 least one alpha receptor subunit and optionally one or more beta, gamma, delta, or epsilon receptor subunit.

This is achieved by co-transfecting cells with multiple expression vectors, each harbouring cDNAs encoding for an α , θ , and optionally one or more β , γ , δ , or ϵ GABA receptor subunits. In a further aspect, therefore, 30 the present invention provides a process for the preparation of a eukaryotic cell line capable of expressing a GABA receptor, which

comprises stably co-transfecting a eukaryotic host cell with at least two expression vectors, one such vector harbouring the cDNA sequence encoding the theta GABA receptor subunit, and another such vector harbouring the cDNA sequence encoding an alpha GABA receptor subunit.

5 The stable cell-line which is established expresses an $\alpha\theta$ GABA receptor.

Each receptor thereby expressed, comprising a unique combination of α , θ and optionally one or more subunits selected from β , γ , δ or ϵ subunits, will be referred to hereinafter as a GABA receptor "subunit combination".

10 Expression of the GABA receptor may be accomplished by a variety of different promoter-expression systems in a variety of different host cells. The eukaryotic host cells suitably include yeast, insect and mammalian cells. Preferably the eukaryotic cells which can provide the host for the expression of the receptor are mammalian cells. Suitable host

15 cells include rodent fibroblast lines, for example mouse Ltk⁻, Chinese hamster ovary (CHO) and baby hamster kidney (BHK); HeLa; and HEK293 cells. It is necessary to incorporate at least one α subunit, the θ subunit, and optionally one or more subunits selected from β , γ , δ or ϵ into the cell line in order to produce the required receptor. Within this

20 limitation, the choice of receptor subunit combination is made according to the type of activity or selectivity which is being screened for.

In order to employ this invention most effectively for screening purposes, it is preferable to build up a library of cell lines, each with a different combination of subunits. Typically a library of 5 or 6 cell line

25 types is convenient for this purpose. Preferred subunit combinations include: $\alpha\theta\beta$, $\alpha\theta\gamma$, $\alpha\theta\delta$, and $\alpha\theta\epsilon$, and most especially $\alpha_1\theta\gamma_2$. Further preferred subunit combinations include $\alpha\beta\theta\gamma$ and $\alpha\beta\theta\epsilon$, and most especially $\alpha_2\beta_1\theta\gamma_1$ and $\alpha_2\beta_3\theta\gamma_2$.

Cells are then co-transfected with the desired combination of the

30 expression vectors. There are several commonly used techniques for transfection of eukaryotic cells *in vitro*. Calcium phosphate precipitation

of DNA is most commonly used (Bachetti *et al.*, *Proc. Natl. Acad. Sci. USA*, 1977, 74, 1590-1594; Maitland *et al.*, *Cell*, 1977, 14, 133-141), and represents a favoured technique in the context of the present invention.

A small percentage of the host cells takes up the recombinant DNA.
5 In a small percentage of those, the DNA will integrate into the host cell chromosome. Because an antibiotic resistance marker gene, such as the neomycin or zeocin resistance gene, will have been incorporated into these host cells, they can be selected by isolating the individual clones which will grow in the presence of the chosen antibiotic, e.g. neomycin or zeocin.
10 Each such clone may then be tested to identify those which will produce the receptor. This may be achieved by inducing the production, for example with dexamethasone, and then detecting the presence of receptor by means of radioligand binding.

Alternatively, expression of the GABA receptor may be effected in
15 *Xenopus* oocytes (see, for instance, Hadingham *et al. Mol. Pharmacol.*, 1993, 44, 1211-1218). Briefly, isolated oocyte nuclei are injected directly with injection buffer or sterile water containing at least one alpha subunit, the theta subunit, and optionally one or more beta, gamma, delta or epsilon receptor subunits, engineered into a suitable expression vector.
20 The oocytes are then incubated.

The expression of subunit combinations in the transfected oocytes may be demonstrated using conventional patch clamp assay. This assay measures the charge flow into and out of an electrode sealed on the surface of the cell. The flow of chloride ions entering the cell *via* the
25 GABA gated ion channel is measured as a function of the current that leaves the cell to maintain electrical equilibrium within the cell as the gate opens.

In a further aspect, the present invention provides protein preparations of GABA receptor subunit combinations, especially human
30 GABA receptor subunit combinations, derived from cultures of stably transfected eukaryotic cells.

The protein preparations of GABA receptor subunit combinations can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

The GABA theta subunit polypeptide of the present invention is also useful for identifying other subunits of the GABA receptor. An example of a procedure for identifying these subunits comprises raising high titre polyclonal antisera against unique, bacterially expressed GABA theta polypeptides. These polyclonal antisera are then used to immunoprecipitate detergent-solubilized GABA receptors from a mammalian brain, for example, a rat brain.

The invention also provides preparations of membranes containing subunit combinations of the GABA receptor, especially human GABA receptor subunit combinations, derived from cultures of stably transfected eukaryotic cells.

The cell line, and the membrane preparations therefrom, according to the present invention have utility in screening and design of drugs

which act upon the GABA receptor, for example benzodiazepines, barbiturates, β -carboline and neurosteroids.

Receptor localisation studies using *in situ* hybridization in monkey brains shows that the θ subunit has a restricted localisation; residing
5 mainly in components of the limbic system (involved in emotions such as rage, fear, motivation sexual behaviours and feeding); medial septum, cingulate cortex, the amygdala and hippocampal fields, in various hypothalamic nuclei, and in regions that have been associated with pain
10 perception; the cingulate cortex, insular cortex, and in mid brain and pons structures.

The present invention accordingly provides the use of stably cotransfected cell lines described above, and membrane preparations derived therefrom, in screening for and designing medicaments which act upon GABA receptors comprising the θ subunit. Of particular interest in
15 this context are molecules capable of interacting selectively with GABA receptors made up of varying subunit combinations. As will be readily apparent, the cell line in accordance with the present invention, and the membrane preparations derived therefrom, provide ideal systems for the study of structure, pharmacology and function of the various GABA
20 receptor subtypes. In particular, preferred screens are functional assays utilizing the pharmacological properties of the GABA receptor subunit combinations of the present invention.

Thus, according to a further aspect of the present invention, there is provided a method for determining whether a ligand, not known to be
25 capable of binding to a human GABA_A receptor comprising the theta subunit, can bind to a human GABA_A receptor comprising the theta subunit, which comprises contacting a mammalian cell comprising DNA molecules encoding at least one alpha receptor subunit, the theta receptor subunit, and optionally one or more beta, gamma, delta or epsilon receptor
30 subunits with the ligand under conditions permitting binding of ligands known to bind to the GABA_A receptor, detecting the presence of any of the

ligand bound to the GABA_A receptor comprising the theta subunit, and thereby determining whether the ligand binds to the GABA_A receptor comprising the theta subunit. The theta subunit-encoding DNA in the cell may have a coding sequence substantially the same as the coding
5 sequence shown in Figure 1 or Figure 2. Preferably, the mammalian cell is non-neuronal in origin. An example of a non-neuronal mammalian cell is a fibroblast cell such as an Ltk⁻ cell. The preferred method for determining whether a ligand is capable of binding to a human GABA_A receptor comprising the theta subunit comprises contacting a transfected
10 non-neuronal mammalian cell (i.e. a cell that does not naturally express any type of GABA_A receptor, and thus will only express such a receptor if it is transfected into the cell) expressing a GABA_A receptor comprising the theta subunit on its surface, or contacting a membrane preparation from such a transfected cell, with the ligand under conditions which are known
15 to prevail, and thus to be associated with, *in vivo* binding of the ligands to a GABA_A receptor comprising the theta subunit, detecting the presence of any of the ligand being tested bound to the GABA_A receptor comprising the theta subunit on the surface of the cell, and thereby determining whether the ligand binds to a human GABA_A receptor comprising the
20 theta subunit. This response system may be based on ion flux changes measured, for example, by scintillation counting (where the ion is radiolabelled) or by interaction of the ion with a fluorescent marker. Particularly suitable ions are chloride ions. Such a host system is conveniently isolated from pre-existing cell lines. Such a transfection
25 system provides a complete response system for investigation or assay of the activity of human GABA_A receptors comprising the theta subunit with ligands as described above. Transfection systems are useful as living cell cultures for competitive binding assays between known or candidate drugs and ligands which bind to the receptor and which are labeled by
30 radioactive, spectroscopic or other reagents. Membrane preparations containing the receptor isolated from transfected cells are also useful for

these competitive binding assays. A transfection system constitutes a “drug discovery system” useful for the identification of natural or synthetic compounds with potential for drug development that can be further modified or used directly as therapeutic compounds to activate, inhibit or
5 modulate the natural functions of human GABA_A receptors comprising the theta subunit. The transfection system is also useful for determining the affinity and efficacy of known drugs at human GABA_A receptor sites comprising the theta subunit.

This invention also provides a method of screening drugs to identify
10 drugs which specifically interact with, and bind to, a human GABA_A receptor comprising the theta subunit on the surface of a cell which comprises contacting a mammalian cell comprising DNA molecules encoding at least one alpha receptor subunit, the theta receptor subunit and optionally one or more beta, gamma, delta or epsilon receptor
15 subunits on the surface of a cell with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, human GABA_A receptors comprising the theta subunit. The theta subunit-encoding DNA in the cell may have a coding sequence substantially the same as the
20 coding sequence shown in Figure 1 or Figure 2. Preferably, the mammalian cell is non-neuronal in origin. An example of a non-neuronal mammalian cell is a fibroblast cell such as an Ltk⁻ cell. Drug candidates are identified by choosing chemical compounds which bind with high affinity to the expressed GABA_A receptor protein in transfected cells,
25 using radioligand binding methods well known in the art. Drug candidates are also screened for selectivity by identifying compounds which bind with high affinity to one particular GABA_A receptor combination but do not bind with high affinity to any other GABA_A receptor combination or to any other known receptor site. Because
30 selective, high affinity compounds interact primarily with the target GABA_A receptor site after administration to the patient, the chances of

producing a drug with unwanted side effects are minimized by this approach.

In the above screens, the mammalian cell may, for example, comprise DNA molecules encoding at least one alpha receptor subunit, the theta subunit, and optionally one or more gamma receptor subunits and optionally one or more beta receptor subunits.

More preferably, in the above screens, the mammalian cell comprises DNA molecules encoding at least one alpha receptor subunit, at least one gamma receptor subunit and the theta receptor subunit.

Ligands or drug candidates identified above may be agonists or antagonists at human GABA_A receptors comprising the theta subunit, or may be agents which allosterically modulate a human GABA_A receptor comprising the theta subunit. These ligands or drug candidates identified above may be employed as therapeutic agents, for example, for the modulation of emotions such as rage and fear, of sexual and appetite behaviours and of pain perception.

The ligands or drug candidates of the present invention thus identified as therapeutic agents may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the agonist or antagonist, and a pharmaceutically acceptable carrier or excipient.

Preferably the compositions containing the ligand or drug candidate identified according to the methods of the present invention are in unit dosage forms such as tablets, pills, capsules, wafers and the like. Additionally, the therapeutic agent may be presented as granules or powders for extemporaneous formulation as volume defined solutions or suspensions. Alternatively, the therapeutic agent may be presented in ready-prepared volume defined solutions or suspensions. Preferred forms are tablets and capsules.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical carrier, e.g. conventional

tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other pharmaceutical diluents, e.g. water, to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention, or a non-toxic pharmaceutically acceptable salt thereof. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules.

10 This solid preformulation composition is then subdivided into unit dosage forms of the type described above containing from 0.1 to about 500 mg of the active ingredient of the present invention. The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, 15 the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of 20 materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally include aqueous 25 solutions, suitably flavoured syrups, aqueous or oil suspensions, and flavoured emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, peanut oil or soybean oil, as well as elixirs and similar pharmaceutical vehicles. Suitable dispersing or suspending agents for aqueous suspensions include synthetic and natural gums such as 30 tragacanth, acacia, alginate, dextran, sodium carboxymethylcellulose, methylcellulose, polyvinyl-pyrrolidone or gelatin.

Compositions of the present invention may also be administered via the buccal cavity using conventional technology, for example, absorption wafers.

5 Compositions in the form of tablets, pills, capsules or wafers for oral administration are particularly preferred.

A minimum dosage level for the ligand or drug candidate identified according to the methods of the present invention is about 0.05mg per day, preferably about 0.5mg per day and especially about 2.5mg per day. A maximum dosage level for the ligand or drug candidate is about 3000mg
10 per day, preferably about 1500mg per day and especially about 500mg per day. The compounds are administered on a regimen of 1 to 4 times daily, preferably once or twice daily, and especially once a day.

It will be appreciated that the amount of the therapeutic agent required for use therapy will vary not only with the particular compounds
15 or compositions selected but also with the route of administration, the nature of the condition being treated, and the age and condition of the patient, and will ultimately be at the discretion of the patient's physician or pharmacist.

20 DESCRIPTION OF FIGURES

Figure 1: Nucleotide sequence for the theta subunit, together with its deduced amino acid sequence corresponding thereto
(SEQ.ID.NO.1 and SEQ.ID.NO.2, respectively)

25

Figure 2: Preferred nucleotide sequence for the theta subunit, together with its deduced amino acid sequence corresponding thereto
(SEQ.ID.NO.3 and SEQ.ID.NO.4, respectively).

Figure 3: GABA dose-response curves on HEK cells transiently transfected with and without θ subunit-containing GABA-A receptors ($\alpha_2\beta_1\theta\gamma_1$ and $\alpha_2\beta_1\gamma_1$).

5 The following non-limiting Examples illustrate the present invention.

EXAMPLE 1

10 ISOLATION AND SEQUENCING OF A cDNA ENCODING THE HUMAN GABA_A RECEPTOR θ SUBUNIT.

The Genbank database was searched with GABA_A receptor polypeptide amino acid sequences using the BLAST searching algorithm, and a number of homologous sequences identified. One of these U47334 was investigated in more detail. U47334 contained sequences homologous to part of the amino-terminal extracellular domain and the TM4 spanning domain of other GABA_A receptor subunits, but did not appear to contain any sequence homologous to the regions spanning these domains.

15 Polymerase chain reaction (PCR) was performed to determine if the size of the U47334 sequence was correct, or was for example, the result of an incorrect splicing event. For PCR, a sense (5' gcaaatgaagctgtggttc 3') (SEQ.ID.NO. 5) and antisense (5' caatggtgaacaacccaaag 3') (SEQ.ID.NO. 6) primer were generated from the U47334 sequence, and PCR performed using standard conditions (Whiting et al, PNAS) using human whole brain cDNA (Clontech) as a template. A second PCR reaction was then performed using nested sense (5' gcctgagaccgaattttgg 3') (SEQ.ID.NO. 7) and antisense (5' ggaaccgggaccactgttc 3') (SEQ.ID.NO. 8) primers generated from the U47334 sequence, and using the products from the first PCR as a template. A single PCR product of approximately 1600 bp was obtained suggesting that the U47334 sequence represents an

20
25
30

incorrectly processed message. This product was sequenced directly using an Applied Biosystems 373 DNA sequencer and dye terminator chemistry.

cDNA sequences 5' and 3' of the U47334 sequence were obtained by 5'- and 3'-anchored PCR using human brain Marathon cDNA cloning kit (Clontech) according to the manufacturer's protocols. The nested antisense (5' tagtccagggtcaagttc 3' and 5' tagtatgctaagcgtgaatc 3') (SEQ.ID.NOS. 9 and 10) and sense (5' gagtttgaggatagttgc 3' and 5' tgctccttactgaaggg 3') (SEQ.ID.NOS. 11 and 12) primers were derived from both the U47334 sequence and the sequence from the initial PCR amplifications. The PCR products were sequenced directly as previously described.

A full length cDNA was generated by PCR using primers derived from sequences downstream of the initiating ATG (5' ccatgactcaagcttgccaccatgctgagccgcagtgatc 3', incorporating a HindIII site) (SEQ.ID.NO. 13) and in the 3' UT of the anchored PCR product (5' tgaaaggagcacagcacagtgtcccg 3') (SEQ.ID.NO. 14). The PCR product (1958 bp) was cloned into pMOS (Amersham), subcloned into pCDNAI Amp (Invitrogen), and sequenced completely on both strands by primer walking. Sequence analysis was performed using Inherit (Applied Biosystems), Sequencher (Genecodes), and Genetics Computer Group (Univ. Wisconsin) computer programs.

The coding region encodes 627 amino acids and has all the structural motifs expected of a ligand gated ion channel subunit. Comparison with other ligand gated ion channel subunits indicates that it is most similar to GABA_A receptor subunits, the highest homology being with the β_1 subunit (45 % identity). However, this sequence identity is sufficiently low as to indicate that the new subunit cannot be classified as a fourth human β subunit, but represents a novel class of subunit, classified as θ , within the GABA receptor gene family.

EXAMPLE 2**LOCALISATION OF THE θ SUBUNIT IN MONKEY BRAIN BY *IN SITU* HYBRIDISATION.**

5

Antisense oligonucleotide probes to the human θ subunit sequence were generated on an Applied Biosystems Automated DNA synthesiser

Probe 1

10 5' CTG-CTT-CTT-GCA-CAC-CCT-TCT-CGC-CAT-GGT-GAA-GCA-TGG-GCT-TCC 3' (SEQ.ID.NO. 15)

Probe 2

5'TGT-CGC-CTA-GGC-TGG-CGC-CGA-GGT-CCT-CGA-CTG-TAG-AAA-AGA-TAG 3' (SEQ.ID.NO. 16)

15 Each oligonucleotide was 3'-end labelled with [³⁵S] deoxyadenosine 5'-(thiotriphosphate) in a 30:1 molar ratio of ³⁵S-isotope:oligonucleotide using terminal deoxynucleotidyl transferase for 15 min at 37°C in the reaction buffer supplied. Radiolabelled oligonucleotide was separated from unincorporated nucleotides using Sephadex G50 spin columns. The

20 specific activities of the labelled probes in several labelling reactions varied from 1.2-2.3 x 10⁹ cpm/mg. Monkey brains were removed and fresh frozen in 1 cm blocks. 12 μ m sections were taken and fixed for *in situ* hybridisation. Hybridisation of the sections was carried out according to the method of Sirinathsingji and Dunnett (Imaging gene expression in

25 neural graft; *Molecular Imaging in Neuroscience: A Practical Approach*, N.A. Sharif (ed), Oxford University Press, Oxford, pp43-70, 1993). Briefly, sections were removed from alcohol, air dried and 3 x10⁵ cpm of each ³⁵S-labelled probe in 100 μ l of hybridisation buffer was applied to each slide. Labelled "antisense" probe was also used in the presence of an

30 excess (100x) concentration of unlabelled antisense probe to define non-specific hybridisation. Parafilm coverslips were placed over the sections

which were incubated overnight (about 16 hr) at 37°C. Following hybridisation the sections were washed for 1 hr at 57°C in 1xSSC then rinsed briefly in 0.1 x SSC, dehydrated in a series of alcohols, air dried and exposed to Amersham Hyperfilm β max X-ray film and the relative
5 distribution of the mRNA assessed for a variety of brain regions.

Messenger RNA for the subunit was seen in components of the limbic system (involved in emotions such as rage, fear, motivation sexual behaviours and feeding) ; medial septum, cingulate cortex, the amygdala and hippocampal fields (dentate gyrus, CA3, CA2, CA1) and in various
10 hypothalamic nuclei (often associated with the limbic system). Messenger RNA was also present in regions that have been associated with pain perception; the cingulate cortex, insular cortex, and in mid brain and pons structures (e.g. central grey and reticular formation) .

15 EXAMPLE 3

LOCALISATION OF THE θ SUBUNIT IN MONKEY BRAIN BY WESTERN BLOT ANALYSIS AND IMMUNOCYTOCHEMISTRY

20

Antibodies to the human GABA_A Theta subunit were generated by sub-cutaneous injection of two New Zealand White rabbits with a glutathione-S-transferase (GST) fusion protein consisting of residues 353-595 of the large cytoplasmic loop region of the theta subunit. DNA
25 encoding this region was cloned into the bacterial expression vector pGEX-2T (Pharmacia), transformed into *E. coli* DH10B cells (Life Technologies), and expression of the fusion protein was carried out using the Pharmacia protocols. The bacterial cells were incubated on ice in STE solution (150 mM NaCl, 10 mM Tris-HCl pH 8, 1 mM EDTA) containing 100 μ g/ml
30 Lysozyme for 20 min before the addition of N-lauryl sarkosine to 1.5 % (w/v). The bacterial slurry was sonicated on ice, and any insoluble matter

removed by centrifugation. Triton X-100 was added to 3 % (v/v) final and the GST-fusion protein purified by glutathione-agarose affinity chromatography. Columns were washed extensively with PBS and the bound protein eluted with 20 mM free glutathione in 150 mM NaCl, 100 mM Tris-HCl pH 9, 1 mM EDTA, 1 mM Dithiothreitol. Eluted protein was concentrated by precipitation with 5 volumes of cold acetone, resuspended in water, and stored at -70 °C until use.

For western blot analysis tissue samples were removed and dissected out on a glass plate at 4°C. The tissue was homogenised in 50mM Tris, pH 7.5, containing 1mM PMSF, 1µM pepstatin A. The homogenate was centrifuged (2000 X g) for 10 minutes and the supernatant was centrifuged at 20,000 X g for 45 minutes. The pellet was resuspended in 50mM Tris and recentrifuged. The final pellet was resuspended in 50mM Tris pH 7.4 containing protease inhibitors and detergent (Na-deoxycholate:0.25%, 150mM NaCl, 1mM EDTA, 1mM PMSF, 1µM pepstatin and leupeptin. Membrane preparations were separated on a 10 % Tris tricine polyacrylamide gel and electrophoretically transferred to nitrocellulose. Nitrocellulose was blocked with 5% non-fat milk (marvel™)/PBS/Tween (0.5%) for 1 hour at room temperature. The anti θ subunit antibody was used at a concentration of 1:500 made up in PBS/Tween/milk at 4°C overnight, washed and then incubated with anti-rabbit IgG HRP linked (Amersham) at 1:1000 in PBS/Tween/milk for one hour at room temperature. The filters were washed, incubated in ECL (Amersham) for 1min and exposed to film. A single band of approximately 60-66kDa was visualised in brainstem and striatal membranes, close to the predicted molecular weight for the θ subunit of 68-74 kDa.

For localisation of the θ subunit by immunocytochemistry a rhesus monkey was deeply anaesthetised with ketamine and sodium pentobarbitone and transcardially perfused with saline, followed by 10% formal saline. The brain was removed, post fixed for 24 hours, and sliced

into coronal blocks, which were then dehydrated through graded alcohols, cleared and embedded in paraffin wax. Coronal sections (8µm) were cut on a base sledge microtome and mounted on glass microscope slides. Sections were deparaffinised, rehydrated and rinsed in 0.1M phosphate buffered saline (PBS). In order to enhance the immunoreactivity sections were subjected to antigen retrieval techniques. Briefly, sections were placed in 0.1M citrate buffer pH 6.0 and given two 5 minute bursts at full power in a conventional microwave oven (800W). Once rinsed in PBS, sections were incubated in 5% normal goat serum in PBS, for 1 hr to block background staining. Sections were then incubated overnight at +4°C in the anti θ subunit rabbit polyclonal antibody (1:1000 diluted in blocking buffer). Immunoreactivity was visualised using the Vector *elite*TM system (Vector Laboratories, Peterborough, U.K.), followed by development in diaminobenzidine (DAB) (Sigma, U.K.). Sections were counterstained in Gill's haematoxylin (Biomen, High Wycombe, U.K.), dehydrated and mounted for microscopical examination. For comparison, samples of 10% formalin immersion fixed post mortem human brainstem were processed in an identical manner. Comparable sections were used to detect θ subunit and tyrosine hydroxylase (Institut Jacques Boy, Reims, France) immunoreactivity by the application of ³⁵S-labeled goat anti rabbit immunoglobulin 1:100 (Amersham Life Sciences, U.K.) for 1 hr. Slides were rinsed in distilled water, dehydrated to 95% ethanol, air dried and exposed to Amersham Hyperfilm β max. Sections used for the immunofluorescent colocalisation of θ subunit and tyrosine hydroxylase were pretreated in the same manner, anti θ subunit immunoreactivity was detected using firstly a biotinylated anti rabbit ;1:200 (Vector Laboratories) followed by FITC conjugated streptavidin (Sigma, U.K.). The second rabbit polyclonal serum, anti tyrosine hydroxylase, was again visualised using biotinylated anti rabbit, reacted with Cy3 conjugated strepavidin (Sigma, U.K.). Sections were counterstained with Hoescht

33258 (0.5 μ g/ml). To avoid any crossreactivity of the detection systems, sections were placed in boiling distilled water for 5 minutes prior to the application of the second primary antibody and its subsequent detection.. The distribution of the θ subunit immunoreactivity in monkey brain

5 reflected the distribution of the θ mRNA observed by *in situ* hybridisation studies (Example 2). Labelled neurons were observed of hypothalamic and cortical pyramidal neurones. Significant labelling was observed of cells in the brainstem, including the substantia nigra pars compacta, ventral and lateral tegmental areas, pigmented neurones of the locus coeruleus and

10 restricted population within the dorsal raphe. Labelling of cell terminals within the caudate putamen was also observed. This distribution was found to closely resemble the distribution of tyrosine hydroxylase immunoreactivity, a marker of catecholaminergic neurones and their processes, visualised by immunautoradiography. θ subunit colocalisation

15 with tyrosine hydroxylase containing neurons was confirmed, using combination immunofluorescence. The expression of the θ subunit seen in both the catecholaminergic neurons of the substantia nigra pars compacta and locus coeruleus was further substantiated in sections of human post mortem brainstem.

20

EXAMPLE 4

CONSTRUCTION OF AN LTK- CELL LINE EXPRESSING THE THETA RECEPTOR SUBUNIT

25

A chimeric construct of the theta subunit was constructed in the mammalian expression vector pcDNA3.1Zeo (Invitrogen) that consisted of bases -224 to +99 of bovine GABA_A α 1 gene, a sequence encoding the c-

30 myc epitope tag (residues 410-419 of the human oncogene product c-myc), a cloning site encoding the amino acids aparagine - serine - glycine, and

DNA encoding residues 22-627 of the GABA_A θ gene product. This construct was linearised and the DNA transfected into a clonal population of mouse Ltk⁻ cells that had previously been shown to be stably transfected with the GABA_A receptor subunits $\alpha_2\beta_1\gamma_1$ and separately an Ltk⁻ line stably transfected with $\alpha_2\beta_3\gamma_2$. The resultant cells were clonally selected with Zeocin selection (100 μ g/ml), and screened to verify stable integration and expression of $\alpha_2\beta_1\theta\gamma_1$ and $\alpha_2\beta_3\theta\gamma_2$ respectively.

EXAMPLE 5

10

WHOLE CELL PATCH-CLAMP OF HEK 293 CELLS TRANSIENTLY TRANSFECTED WITH HUMAN GABA-A RECEPTORS

15

Experiments were performed on HEK 293 cells transiently transfected with human cDNA combinations $\alpha_2\beta_1\gamma_1$, and $\alpha_2\beta_1\theta\gamma_1$ (4 μ gs of cDNA total per cover-slip) using calcium phosphate precipitation (Chen and Okayama, 1988) as previously described (Hadingham et al, 1993). Glass cover-slips containing the cells in a monolayer culture were transferred to a perspex chamber on the stage of Nikon Diaphot inverted microscope. Cells were continuously perfused with a solution containing 124mM NaCl, 2mM KCl, 2mM CaCl₂, 1mM MgCl₂, 1.25mM KH₂PO₄, 25mM NaHCO₃, 11mM D-glucose, at pH 7.2, and observed using phase-contrast optics. Patch-pipettes were pulled with an approximate tip diameter of 2 μ m and a resistance of 4M Ω with borosilicate glass and filled with 130mM CsCl, 10mM HEPES, 10mM EGTA, 3mM Mg⁺-ATP, pH adjusted to 7.3 with CsOH. Cells were patch-clamped in whole-cell mode using an Axopatch 200B patch-clamp amplifier. Drug solutions were applied by a double-barrelled pipette assembly, controlled by a stepping motor attached to a Prior manipulator, enabling rapid equilibration

30

around the cell. Increasing GABA concentrations were applied for 2sec pulses with a 30sec interval between applications. Non-cumulative concentration-response curves to GABA were constructed. Curves were fitted using a non-linear square-fitting program to the equation $f(x) =$
 5 $B_{MAX}/[1+(EC_{50}/x)^n]$ where x is the drug concentration, EC_{50} is the concentration of drug eliciting a half-maximal response and n is the Hill coefficient. EC_{50} 's were analysed by unpaired students t-test.

The GABA EC_{50} of HEK 293 cells transiently expressing the GABA_A receptor subunit combination $\alpha_2\beta_1\theta\gamma_1$ is significantly lower than that of
 10 HEK 293 cells transiently expressing the GABA_A receptor subunit combination $\alpha_2\beta_1\gamma_1$ (see Figure 3).

	$\alpha_2\beta_1\gamma_1$	$\alpha_2\beta_1\theta\gamma_1$
EC_{50}	16.7±3.7 nM	62.7±6.7 nM*
Slope	1.6±0.2	1.5±0.1

* p<0.001

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Merck Sharp & Dohme Limited
- (B) STREET: Terlings Park, Eastwick Road
- (C) CITY: Harlow
- (D) STATE: Essex
- (E) COUNTRY: England
- (F) POSTAL CODE (ZIP): CM20 2QR
- (G) TELEPHONE: +44 1279 440175
- (H) TELEFAX: +44 1279 440717

(ii) TITLE OF INVENTION: Human theta subunit of the GABA-A receptor

(iii) NUMBER OF SEQUENCES: 16

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1884 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

```

ATGCTGCGAG CCGCAGTGAT CCTGCTGCTC ATCAGGACCT GGCTCGCGGA GGGCAACTAC 60
CCCAGTCCCA TCCCGAAATT CCACTTCGAG TTCTCCTCTG CTGTGCCCGA AGTCGTCCTG 120
AACCTCTTCA ACTGCAAAAA TTGTGCAAAT GAAGCTGTGG TTCAAAGAT TTTGGACAGG 180
GTGCTGTCAA GATACGATGT CCGCCTGAGA CCGAATTTTG GAGGTGCCCC TGTGCCTGTG 240
AGAATATCTA TTTATGTCAC GAGCATTGAA CAGATCTCAG AAATGAATAT GGACTACACG 300
ATCACGATGT TTTTTCATCA GACTTGAAA GATTCACGCT TAGCATACTA TGAGACCACC 360

```

CTGAACTTGA CCCTGGACTA TCGGATGCAT GAGAAGTTGT GGGTCCCTGA CTGCTACTTT 420
 CTGAACAGCA AGGATGCTTT CGTGCATGAT GTGACTGTGG AGAATCGCGT GTTTCAGCTT 480
 CACCCAGATG GAACGGTGCG GTACGGCATC CCACTCACCA CTACAGCAGT TTGTTCCCTG 540
 GATCTGCATA AATTCCCTAT GGACAAGCAG GCCTGCAACC TGGTGGTAGA GAGCTATGGT 600
 TACACGGTTG AAGACATCAT ATTATTCTGG GATGACAATG GGAACGCCAT CCACATGACT 660
 GAGGAGCTGC ATATCCCTCA GTTCACTTTC CTGGGAAGGA CGATTACTAG CAAGGAGGTG 720
 TATTTCTACA CAGGTTCCCTA CATAACGCTG AACTGGAAGT TCCAGGTTCA GAGGGAAGTT 780
 AACAGCTACC TTGTGCAAGT CTAAGTGCCT ACTGTCCTCA CCACTATTAC CTCTTGATA 840
 TCGTTTTGGA TGAAGTATGA TTCCTCTGCA GCCAGGGTGA CAATTGGCTT AACTTCAATG 900
 CTCATCCTGA CCACCATCGA CTCACATCTG CGGGATAAGC TCCCAACAT TTCCTGTATC 960
 AAGGCCATTG ATATCTATAT CCTCGTGTGC TTGTTCTTTG TGTTCTGTC CTTGCTGGAG 1020
 TATGTCTACA TCAACTATCT TTTCTACAGT CGAGGACCTC GGCGCCAGCC TAGGCGACGC 1080
 AGGAGACCCC GAAGAGTCAT TGCCCGCTAC CGCTACCAGC AAGTGGTGGT AGGAAACGTG 1140
 CAGGATGGCC TGATTAACGT GGAAGACGGA GTCAGCTCTC TCCCCATCAC CCCAGCGCAG 1200
 GCCCCCTGG CAAGCCCGGA AAGCCTCGGT TCTTTGACGT CCACCTCCGA GCAGGCCAG 1260
 CTGGCCACCT CGGAAAGCCT CAGCCACTC ACTTCTCTCT CAGGCCAGGC CCCCCTGGCC 1320
 ACTGGAGAAA GCCTGAGCGA TCTCCCCTCC ACCTCAGAGC AGGCCCGGCA CAGCTATGGT 1380
 GTTCGCTTTA ATGGTTTCCA GGCTGATGAC AGTATTATTC CTACCGAAAT CCGCAACCGT 1440
 GTCGAAGCCC ATGGCCATGG TGTTACCCAT GACCATGAAG ATTCCAATGA GAGCTTGAGC 1500
 TCGGATGAGC GCCATGGCCA TGGCCCCAGT GGAAGCCCA TGCTTCACCA TGGCGAGAAG 1560
 GGTGTGCAAG AAGCAGGCTG GGACCTTGAT GACAACAATG ACAAGAGCGA CTGCCTTGCC 1620
 ATTAAGGAGC AATTCAAGTG TGATACTAAC AGTACCTGGG GCCTTAATGA TGATGAGCTC 1680
 GTGGCCCATG GCCAAGAGAA GGACAGTAGC TCAGAGTCTG AGGATAGTTG CCCCCAAGC 1740
 CCTGGGTGCT CCTTCACTGA AGGGTTCTCC TTCGATCTCT TTAATCCTGA CTACGTCCCA 1800
 AAGGTCGACA AGTGGTCCCG GTTCCTCTTC CCTCTGGCCT TTGGGTTGTT CAACATTGTT 1860
 TACTGGGTAT ACCATATGTA TTAG 1884

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 627 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Met	Leu	Arg	Ala	Ala	Val	Ile	Leu	Leu	Leu	Ile	Arg	Thr	Trp	Leu	Ala	1	5	10	15
Glu	Gly	Asn	Tyr	Pro	Ser	Pro	Ile	Pro	Lys	Phe	His	Phe	Glu	Phe	Ser	20	25	30	
Ser	Ala	Val	Pro	Glu	Val	Val	Leu	Asn	Leu	Phe	Asn	Cys	Lys	Asn	Cys	35	40	45	
Ala	Asn	Glu	Ala	Val	Val	Gln	Lys	Ile	Leu	Asp	Arg	Val	Leu	Ser	Arg	50	55	60	
Tyr	Asp	Val	Arg	Leu	Arg	Pro	Asn	Phe	Gly	Gly	Ala	Pro	Val	Pro	Val	65	70	75	80
Arg	Ile	Ser	Ile	Tyr	Val	Thr	Ser	Ile	Glu	Gln	Ile	Ser	Glu	Met	Asn	85	90	95	
Met	Asp	Tyr	Thr	Ile	Thr	Met	Phe	Phe	His	Gln	Thr	Trp	Lys	Asp	Ser	100	105	110	
Arg	Leu	Ala	Tyr	Tyr	Glu	Thr	Thr	Leu	Asn	Leu	Thr	Leu	Asp	Tyr	Arg	115	120	125	
Met	His	Glu	Lys	Leu	Trp	Val	Pro	Asp	Cys	Tyr	Phe	Leu	Asn	Ser	Lys	130	135	140	
Asp	Ala	Phe	Val	His	Asp	Val	Thr	Val	Glu	Asn	Arg	Val	Phe	Gln	Leu	145	150	155	160
His	Pro	Asp	Gly	Thr	Val	Arg	Tyr	Gly	Ile	Arg	Leu	Thr	Thr	Thr	Ala	165	170	175	
Val	Cys	Ser	Leu	Asp	Leu	His	Lys	Phe	Pro	Met	Asp	Lys	Gln	Ala	Cys	180	185	190	
Asn	Leu	Val	Val	Glu	Ser	Tyr	Gly	Tyr	Thr	Val	Glu	Asp	Ile	Ile	Leu	195	200	205	
Phe	Trp	Asp	Asp	Asn	Gly	Asn	Ala	Ile	His	Met	Thr	Glu	Glu	Leu	His	210	215	220	
Ile	Pro	Gln	Phe	Thr	Phe	Leu	Gly	Arg	Thr	Ile	Thr	Ser	Lys	Glu	Val	225	230	235	240

Tyr Phe Tyr Thr Gly Ser Tyr Ile Arg Leu Ile Leu Lys Phe Gln Val
 245 250 255
 Gln Arg Glu Val Asn Ser Tyr Leu Val Gln Val Tyr Trp Pro Thr Val
 260 265 270
 Leu Thr Thr Ile Thr Ser Trp Ile Ser Phe Trp Met Asn Tyr Asp Ser
 275 280 285
 Ser Ala Ala Arg Val Thr Ile Gly Leu Thr Ser Met Leu Ile Leu Thr
 290 295 300
 Thr Ile Asp Ser His Leu Arg Asp Lys Leu Pro Asn Ile Ser Cys Ile
 305 310 315 320
 Lys Ala Ile Asp Ile Tyr Ile Leu Val Cys Leu Phe Phe Val Phe Leu
 325 330 335
 Ser Leu Leu Glu Tyr Val Tyr Ile Asn Tyr Leu Phe Tyr Ser Arg Gly
 340 345 350
 Pro Arg Arg Gln Pro Arg Arg Arg Arg Arg Pro Arg Arg Val Ile Ala
 355 360 365
 Arg Tyr Arg Tyr Gln Gln Val Val Val Gly Asn Val Gln Asp Gly Leu
 370 375 380
 Ile Asn Val Glu Asp Gly Val Ser Ser Leu Pro Ile Thr Pro Ala Gln
 385 390 395 400
 Ala Pro Leu Ala Ser Pro Glu Ser Leu Gly Ser Leu Thr Ser Thr Ser
 405 410 415
 Glu Gln Ala Gln Leu Ala Thr Ser Glu Ser Leu Ser Pro Leu Thr Ser
 420 425 430
 Leu Ser Gly Gln Ala Pro Leu Ala Thr Gly Glu Ser Leu Ser Asp Leu
 435 440 445
 Pro Ser Thr Ser Glu Gln Ala Arg His Ser Tyr Gly Val Arg Phe Asn
 450 455 460
 Gly Phe Gln Ala Asp Asp Ser Ile Ile Pro Thr Glu Ile Arg Asn Arg
 465 470 475 480
 Val Glu Ala His Gly His Gly Val Thr His Asp His Glu Asp Ser Asn
 485 490 495
 Glu Ser Leu Ser Ser Asp Glu Arg His Gly His Gly Pro Ser Gly Lys
 500 505 510
 Pro Met Leu His His Gly Glu Lys Gly Val Gln Glu Ala Gly Trp Asp
 515 520 525
 Leu Asp Asp Asn Asn Asp Lys Ser Asp Cys Leu Ala Ile Lys Glu Gln
 530 535 540

Phe Lys Cys Asp Thr Asn Ser Thr Trp Gly Leu Asn Asp Asp Glu Leu
 545 550 555 560
 Val Ala His Gly Gln Glu Lys Asp Ser Ser Ser Glu Ser Glu Asp Ser
 565 570 575
 Cys Pro Pro Ser Pro Gly Cys Ser Phe Thr Glu Gly Phe Ser Phe Asp
 580 585 590
 Leu Phe Asn Pro Asp Tyr Val Pro Lys Val Asp Lys Trp Ser Arg Phe
 595 600 605
 Leu Phe Pro Leu Ala Phe Gly Leu Phe Asn Ile Val Tyr Trp Val Tyr
 610 615 620
 His Met Tyr
 625

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1884 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

ATGCTGCGAG CCGCAGTGAT CCTGCTGCTC ATCAGGACCT GGCTCGCGGA GGGCAACTAC 60
 CCCAGTCCCA TCCCGAAATT CCACTTCGAG TTCTCCTCTG CTGTGCCCGA AGTCGTCCTG 120
 AACCTCTTCA ACTGCAAAAA TTGTGCAAAT GAAGCTGTGG TTCAAAGAT TTTGGACAGG 180
 GTGCTGTCAA GATACGATGT CCGCCTGAGA CCGAATTTTG GAGGTGCCCC TGTGCCTGTG 240
 AGAATATCTA TTTATGTCAC GAGCATTGAA CAGATCTCAG AAATGAATAT GGACTACACG 300
 ATCACGATGT TTTTTCATCA GACTTGGAAA GATTCACGCT TAGCATACTA TGAGACCACC 360
 CTGAACTTGA CCCTGGACTA TCGGATGCAT GAGAAGTTGT GGGTCCCTGA CTGCTACTTT 420
 TTGAACAGCA AGGATGCTTT CGTGCATGAT GTGACTGTGG AGAATCGCGT GTTTCAGCTT 480
 CACCCAGATG GAACGGTGCG GTACGGCATC CGACTCACCA CTACAGCAGC TTGTTCCCTG 540
 GATCTGCATA AATTCCCTAT GGACAAGCAG GCCTGCAACC TGGTGGTAGA GAGCTATGGT 600
 TACACGGTTG AAGACATCAT ATTATTCTGG GATGACAATG GGAACGCCAT CCACATGACT 660

GAGGAGCTGC ATATCCCTCA GTTCACTTTC CTGGGAAGGA CGATTACTAG CAAGGAGGTG 720
 TATTTCTACA CAGGTTCTTA CATACGCCTG AACTGAAGT TCCAGGTTCA GAGGGAAGTT 780
 AACAGCTACC TTGTGCAAGT CTA CTGGCCT ACTGTCCTCA CCACTATTAC CTCTTGATA 840
 TCGTTTTGGA TGA ACTATGA TTCCTCTGCA GCCAGGGTGA CAATTGGCTT AACTTCAATG 900
 CTCATCCTGA CCACCATCGA CTCACATCTG CGGGATAAGC TCCCCAACAT TTCCTGTATC 960
 AAGGCCATTG ATATCTATAT CCTCGTGTGC TTGTTCTTTG TGTTCCTGTC CTTGCTGGAG 1020
 TATGTCTACA TCAACTATCT TTTCTACAGT CGAGGACCTC GCGCCAGCC TAGGCGACAC 1080
 AGGAGACCCC GAAGAGTCAT TGCCCGCTAC CGCTACCAGC AAGTGGTGGT AGGAAACGTG 1140
 CAGGATGGCC TGATTAACGT GGAAGACGGA GTCAGCTCTC TCCCCATCAC CCCAGCGCAG 1200
 GCCCCCTGG CAAGCCCGGA AAGCCTCGGT TCTTTGACGT CCACCTCCGA GCAGGCCAG 1260
 CTGGCCACCT CGGAAAGCCT CAGCCCCTC ACTTCTCTCT CAGGCCAGGC CCCCTGGCC 1320
 ACTGGAGAAA GCCTGAGCGA TCTCCCCTCC ACCTCAGAGC AGGCCCGCA CAGCTATGGT 1380
 GTTCGCTTTA ATGGTTTCCA GGCTGATGAC AGTATTTTTT CTACCGAAAT CCGCAACCGT 1440
 GTCGAAGCCC ATGGCCATGG TGTTACCCAT GACCATGAAG ATTCCAATGA GAGCTTGAGC 1500
 TCGGATGAGC GCCATGGCCA TGGCCCCAGT GGGAAGCCCA TGCTTCACCA TGGCGAGAAG 1560
 GGTGTGCAAG AAGCAGGCTG GGACCTTGAT GACAACAATG ACAAGAGCGA CTGCCTTGCC 1620
 ATTAAGGAGC AATTCAAGTG TGATACTAAC AGTACCTGGG GCCTTAATGA TGATGAGCTC 1680
 ATGGCCCATG GCCAAGAGAA GGACAGTAGC TCAGAGTCTG AGGATAGTTG CCCCCAAGC 1740
 CCTGGGTGCT CCTTCACTGA AGGGTTCTCC TTCGATCTCT TTAATCCTGA CTACGTCCCA 1800
 AAGGTCGACA AGTGGTCCCG GTTCCTCTTC CCTCTGGCCT TTGGGTGTT CAACATTGTT 1860
 TACTGGGTAT ACCATATGTA TTAG 1884

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 627 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Met Leu Arg Ala Ala Val Ile Leu Leu Leu Ile Arg Thr Trp Leu Ala
 1 5 10 15
 Glu Gly Asn Tyr Pro Ser Pro Ile Pro Lys Phe His Phe Glu Phe Ser
 20 25 30
 Ser Ala Val Pro Glu Val Val Leu Asn Leu Phe Asn Cys Lys Asn Cys
 35 40 45
 Ala Asn Glu Ala Val Val Gln Lys Ile Leu Asp Arg Val Leu Ser Arg
 50 55 60
 Tyr Asp Val Arg Leu Arg Pro Asn Phe Gly Gly Ala Pro Val Pro Val
 65 70 75 80
 Arg Ile Ser Ile Tyr Val Thr Ser Ile Glu Gln Ile Ser Glu Met Asn
 85 90 95
 Met Asp Tyr Thr Ile Thr Met Phe Phe His Gln Thr Trp Lys Asp Ser
 100 105 110
 Arg Leu Ala Tyr Tyr Glu Thr Thr Leu Asn Leu Thr Leu Asp Tyr Arg
 115 120 125
 Met His Glu Lys Leu Trp Val Pro Asp Cys Tyr Phe Leu Asn Ser Lys
 130 135 140
 Asp Ala Phe Val His Asp Val Thr Val Glu Asn Arg Val Phe Gln Leu
 145 150 155 160
 His Pro Asp Gly Thr Val Arg Tyr Gly Ile Arg Leu Thr Thr Thr Ala
 165 170 175
 Ala Cys Ser Leu Asp Leu His Lys Phe Pro Met Asp Lys Gln Ala Cys
 180 185 190
 Asn Leu Val Val Glu Ser Tyr Gly Tyr Thr Val Glu Asp Ile Ile Leu
 195 200 205
 Phe Trp Asp Asp Asn Gly Asn Ala Ile His Met Thr Glu Glu Leu His
 210 215 220
 Ile Pro Gln Phe Thr Phe Leu Gly Arg Thr Ile Thr Ser Lys Glu Val
 225 230 235 240
 Tyr Phe Tyr Thr Gly Ser Tyr Ile Arg Leu Ile Leu Lys Phe Gln Val
 245 250 255
 Gln Arg Glu Val Asn Ser Tyr Leu Val Gln Val Tyr Trp Pro Thr Val
 260 265 270
 Leu Thr Thr Ile Thr Ser Trp Ile Ser Phe Trp Met Asn Tyr Asp Ser
 275 280 285

Ser Ala Ala Arg Val Thr Ile Gly Leu Thr Ser Met Leu Ile Leu Thr
 290 295 300
 Thr Ile Asp Ser His Leu Arg Asp Lys Leu Pro Asn Ile Ser Cys Ile
 305 310 315 320
 Lys Ala Ile Asp Ile Tyr Ile Leu Val Cys Leu Phe Phe Val Phe Leu
 325 330 335
 Ser Leu Leu Glu Tyr Val Tyr Ile Asn Tyr Leu Phe Tyr Ser Arg Gly
 340 345 350
 Pro Arg Arg Gln Pro Arg Arg His Arg Arg Pro Arg Arg Val Ile Ala
 355 360 365
 Arg Tyr Arg Tyr Gln Gln Val Val Val Gly Asn Val Gln Asp Gly Leu
 370 375 380
 Ile Asn Val Glu Asp Gly Val Ser Ser Leu Pro Ile Thr Pro Ala Gln
 385 390 395 400
 Ala Pro Leu Ala Ser Pro Glu Ser Leu Gly Ser Leu Thr Ser Thr Ser
 405 410 415
 Glu Gln Ala Gln Leu Ala Thr Ser Glu Ser Leu Ser Pro Leu Thr Ser
 420 425 430
 Leu Ser Gly Gln Ala Pro Leu Ala Thr Gly Glu Ser Leu Ser Asp Leu
 435 440 445
 Pro Ser Thr Ser Glu Gln Ala Arg His Ser Tyr Gly Val Arg Phe Asn
 450 455 460
 Gly Phe Gln Ala Asp Asp Ser Ile Phe Pro Thr Glu Ile Arg Asn Arg
 465 470 475 480
 Val Glu Ala His Gly His Gly Val Thr His Asp His Glu Asp Ser Asn
 485 490 495
 Glu Ser Leu Ser Ser Asp Glu Arg His Gly His Gly Pro Ser Gly Lys
 500 505 510
 Pro Met Leu His His Gly Glu Lys Gly Val Gln Glu Ala Gly Trp Asp
 515 520 525
 Leu Asp Asp Asn Asn Asp Lys Ser Asp Cys Leu Ala Ile Lys Glu Gln
 530 535 540
 Phe Lys Cys Asp Thr Asn Ser Thr Trp Gly Leu Asn Asp Asp Glu Leu
 545 550 555 560
 Met Ala His Gly Gln Glu Lys Asp Ser Ser Ser Glu Ser Glu Asp Ser
 565 570 575

(ii) MOLECULE TYPE: cDNA

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

GCCTGAGACC GAATTTTGG

19

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

GGAACCGGGA CCACTTGTC

19

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

TAGTCCAGGG TCAAGTTC

18

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

TAGTATGCTA AGCGTGAATC

20

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

GAGTTTGAGG ATAGTTGC 18

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

TGCTCCTTCA CTGAAGGG 18

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

CCATGACTCA AGCTTGCCAC CATGCTGCGA GCCGCAGTGA TC 42

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs

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

(ii) MOLECULE TYPE: cDNA

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

TGAAAGGAGC ACAGCACAGT GCTCCCG

27

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CTGCTTCTTG CACACCCTTC TCGCCATGGT GAAGCATGGG CTTCC 45

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TGTCGCCTAG GCTGGCGCCG AGGTCCTCGA CTGTAGAAAA GATAG 45

CLAIMS:

1. A stably co-transfected eukaryotic cell line capable of
expressing a GABA receptor, which receptor comprises the theta receptor
5 subunit, at least one alpha receptor subunit and optionally one or more
receptor subunits selected from the beta, gamma, delta and epsilon
subunits.
2. A cell line according to claim 1 which is a rodent fibroblast
10 cell line.
3. A process for the preparation of an eukaryotic cell line
capable of expressing a GABA receptor, which comprises stably co-
transfecting a eukaryotic host cell with at least two expression vectors, one
15 such vector harbouring the cDNA sequence encoding the theta GABA
receptor subunit, another such vector harbouring the cDNA sequence
encoding an alpha GABA receptor subunit, and optionally one or more
additional vectors harbouring the cDNA sequence encoding a beta,
gamma, delta or epsilon GABA receptor subunit.
20
4. A process according to claim 3 wherein the cell line is a
rodent fibroblast cell line.
5. A DNA molecule encoding the theta subunit of the human
25 GABA receptor comprising all or a portion of the sequence depicted in
Figure 1 or Figure 2, or a modified human sequence.
6. A recombinant expression vector comprising the nucleotide
sequence of the human GABA receptor theta subunit together with
30 additional sequences capable of directing the synthesis of the said human

GABA receptor theta subunit in cultures of stably co-transfected eukaryotic cells.

7. A protein preparation of GABA receptor subunit combinations derived from a cell line according to claim 1 or 2.

8. A membrane preparation containing subunit combinations of the GABA receptor derived from a cell line according to claim 1 or 2.

9. A preparation according to claim 7 or 8 wherein the subunit combination derived is the $\alpha_1\theta\gamma_2$, $\alpha_2\beta_1\theta\gamma_1$ or $\alpha_2\beta_3\theta\gamma_2$ subunit combination of the GABA receptor.

10. The use of a cell according to claim 1 or 2 or a membrane preparation derived therefrom in screening for and designing medicaments which act upon a GABA receptor comprising the theta subunit.

11. A method for determining whether a ligand, not known to be capable of binding to a human GABA_A receptor comprising the theta subunit, can bind to a human GABA_A receptor comprising the theta subunit, which comprises contacting a mammalian cell comprising DNA molecules encoding at least one alpha receptor subunit, the theta receptor subunit and optionally one or more beta, gamma, delta or epsilon receptor subunits, with the ligand under conditions permitting binding of ligands known to bind to the GABA_A receptor, detecting the presence of any of the ligand bound to the GABA_A receptor comprising the theta subunit and thereby determining whether the ligand binds to the GABA_A receptor comprising the theta subunit.

12. A method of screening drugs to identify drugs which specifically interact with, and bind to, a human GABA_A receptor

comprising the theta subunit on the surface of a cell which comprises
contacting a mammalian cell comprising a DNA molecule encoding at least
one alpha receptor subunit, the theta receptor subunit and optionally one
or more beta, gamma, delta or epsilon receptor subunits, on the surface of
5 a cell with a plurality of drugs, determining those drugs which bind to the
mammalian cell, and thereby identifying drugs which specifically interact
with, and bind to, human GABA_A receptors comprising the theta subunit.

13. A polynucleotide which hybridizes under stringent conditions
10 to the DNA molecule depicted in Figure 1 or Figure 2.

14. A GABA_A receptor theta subunit polypeptide which has the
deduced amino acid sequence of Figure 1 or Figure 2, or a fragment,
analog or derivative thereof.

15

FIGURE 1**Human θ Subunit**

1	ATGCTGCGAGCCGCGAGTGATCCTGCTGCTCATCAGGACCTGGCTCGCGGAGGGCAACTAC	60
	M L R A A V I L L L I R T W L A E G N Y	
61	CCCAGTCCCATCCCGAAATTCCACTTCGAGTTCTCCTCTGCTGTGCCCGAAGTCGTCCTG	120
	P S P I P K F H F E F S S A V P E V V L	
121	AACCTCTTCAACTGCAAAAATTGTGCAAATGAAGCTGTGGTTCAAAGATTTTGGACAGG	180
	N L F N C K N C A N E A V V Q K I L D R	
181	GTGCTGTCAAGATACGATGTCCGCCTGAGACCGAATTTTGGAGGTGCCCTGTGCCTGTG	240
	V L S R Y D V R L R P N F G G A P V P V	
241	AGAATATCTATTTATGTCACGAGCATTGAACAGATCTCAGAAATGAATATGGACTACACG	300
	R I S I Y V T S I E Q I S E M N M D Y T	
301	ATCACGATGTTTTTTCATCAGACTTGGAAGATTCACGCTTAGCATACTATGAGACCACC	360
	I T M F F H Q T W K D S R L A Y Y E T T	
361	CTGAACTTGACCCTGGACTATCGGATGCATGAGAAGTTGTGGGTCCCTGACTGCTACTTT	420
	L N L T L D Y R M H E K L W V P D C Y F	
421	CTGAACAGCAAGGATGCTTTCGTGCATGATGTGACTGTGGAGAATCGCGTGTTCAGCTT	480
	L N S K D A F V H D V T V E N R V F Q L	
481	CACCCAGATGGAACGGTGCGGTACGGCATCCGACTCACCCTACAGCAGTTTGTTCCCTG	540
	H P D G T V R Y G I R L T T T A V C S L	
541	GATCTGCATAAATTCCTATGGACAAGCAGGCCTGCAACCTGGTGGTAGAGAGCTATGGT	600
	D L H K F P M D K Q A C N L V V E S Y G	
601	TACACGGTTGAAGACATCATATTATTCTGGGATGACAATGGGAACGCCATCCACATGACT	660
	Y T V E D I I L F W D D N G N A I H M T	
661	GAGGAGCTGCATATCCCTCAGTTCACTTTCCTGGGAAGGACGATTACTAGCAAGGAGGTG	720
	E E L H I P Q F T F L G R T I T S K E V	
721	TATTTCTACACAGGTTCCCTACATACGCCTGATACTGAAGTTCAGGTTCCAGAGGGAAGTT	780
	Y F Y T G S Y I R L I L K F Q V Q R E V	
781	AACAGCTACCTTGTGCAAGTCTACTGGCCTACTGTCCTCACCCTATTACCTCTTGGATA	840
	N S Y L V Q V Y W P T V L T T I T S W I	
841	TCGTTTTGGATGAACTATGATTCCTCTGCAGCCAGGGTGACAATTGGCTTAACTTCAATG	900
	S F W M N Y D S S A A R V T I G L T S M	
901	CTCATCCTGACCACCATCGACTCACATCTGCGGGATAAGCTCCCCAACATTTCTGTATC	960
	L I L T T I D S H L R D K L P N I S C I	
961	AAGGCCATTGATATCTATATCCTCGTGTGCTTGTTCCTTTGTGTTCCTGTCCTTGCTGGAG	1020
	K A I D I Y I L V C L F F V F L S L L E	

1021 TATGTCTACATCAACTATCTTTTCTACAGTCGAGGACCTCGGCGCCAGCCTAGGCGACGC 1080
 Y V Y I N Y L F Y S R G P R R Q P R R R

1081 AGGAGACCCCGAAGAGTCATTGCCCGCTACCGCTACCAGCAAGTGGTGGTAGGAAACGTG 1140
 R R P R R V I A R Y R Y Q Q V V V G N V

1141 CAGGATGGCCTGATTAACGTGGAAGACGGAGTCAGCTCTCTCCCCATCACCCCAGCGCAG 1200
 Q D G L I N V E D G V S S L P I T P A Q

1201 GCCCCCTGGCAAGCCCGGAAAGCCTCGGTTCTTTGACGTCCACCTCCGAGCAGGCCAG 1260
 A P L A S P E S L G S L T S T S E Q A Q

1261 CTGGCCACCTCGGAAAGCCTCAGCCCACTCACTTCTCTCTCAGGCCAGGCCCCCTGGCC 1320
 L A T S E S L S P L T S L S G Q A P L A

1321 ACTGGAGAAAGCCTGAGCGATCTCCCCTCCACCTCAGAGCAGGCCCGGCACAGCTATGGT 1380
 T G E S L S D L P S T S E Q A R H S Y G

1381 GTTCGCTTTAATGGTTTCCAGGCTGATGACAGTATTATTCTACCGAAATCCGCAACCGT 1440
 V R F N G F Q A D D S I I P T E I R N R

1441 GTCGAAGCCCATGGCCATGGTGTACCCATGACCATGAAGATTCCAATGAGAGCTTGAGC 1500
 V E A H G H G V T H D H E D S N E S L S

1501 TCGGATGAGCGCCATGGCCATGGCCCCAGTGGGAAGCCCATGCTTCACCATGGCGAGAAG 1560
 S D E R H G H G P S G K P M L H H G E K

1561 GGTGTGCAAGAAGCAGGCTGGGACCTTGATGACAACAATGACAAGAGCGACTGCCTTGCC 1620
 G V Q E A G W D L D D N N D K S D C L A

1621 ATTAAGGAGCAATTCAAGTGTGATACTAACAGTACCTGGGGCCTTAATGATGATGAGCTC 1680
 I K E Q F K C D T N S T W G L N D D E L

1681 GTGGCCCATGGCCAAGAGAAGGACAGTAGCTCAGAGTCTGAGGATAGTTGCCCCCAAGC 1740
 V A H G Q E K D S S S E S E D S C P P S

1741 CCTGGGTGCTCCTTCACTGAAGGGTTCTCCTTCGATCTCTTTAATCCTGACTACGTCCCA 1800
 P G C S F T E G F S F D L F N P D Y V P

1801 AAGGTCGACAAGTGGTCCCGGTTCTCTCCCTCTGGCCTTTGGGTTGTTCAACATTGTT 1860
 K V D K W S R F L F P L A F G L F N I V

1861 TACTGGGTATAACCATATGTATTAG 1884
 Y W V Y H M Y *

FIGURE 2
Human θ Subunit

1	ATGCTGCGAGCCGCGAGTGATCCTGCTGCTCATCAGGACCTGGCTCGCGGAGGGCAACTAC	60
	M L R A A V I L L L I R T W L A E G N Y	
61	CCCAGTCCCATCCCGAAATTCACCTTCGAGTTCTCCTCTGCTGTGCCCGAAGTCGTCCTG	120
	P S P I P K F H F E F S S A V P E V V L	
121	AACCTCTTCAACTGCAAAAATTGTGCAAATGAAGCTGTGGTTCAAAGATTTTGGACAGG	180
	N L F N C K N C A N E A V V Q K I L D R	
181	GTGCTGTCAAGATACGATGTCCGCCTGAGACCGAATTTTGGAGGTGCCCCTGTGCCTGTG	240
	V L S R Y D V R L R P N F G G A P V P V	
241	AGAATATCTATTTATGTCACGAGCATTGAACAGATCTCAGAAATGAATATGGACTACACG	300
	R I S I Y V T S I E Q I S E M N M D Y T	
301	ATCACGATGTTTTTTCATCAGACTTGGAAGATTCACGCTTAGCATACTATGAGACCACC	360
	I T M F F H Q T W K D S R L A Y Y E T T	
361	CTGAACTTGACCCTGGACTATCGGATGCATGAGAAGTTGTGGGTCCCTGACTGCTACTTT	420
	L N L T L D Y R M H E K L W V P D C Y F	
421	TTGAACAGCAAGGATGCTTTCGTGCATGATGTGACTGTGGAGAATCGCGTGTTCAGCTT	480
	L N S K D A F V H D V T V E N R V F Q L	
481	CACCCAGATGGAACGGTGCGGTACGGCATCCGACTCACCACTACAGCAGCTTGTTCCCTG	540
	H P D G T V R Y G I R L T T T A A C S L	
541	GATCTGCATAAATCCCTATGGACAAGCAGGCCTGCAACCTGGTGGTAGAGAGCTATGGT	600
	D L H K F P M D K Q A C N L V V E S Y G	
601	TACACGGTTGAAGACATCATATTATTCTGGGATGACAATGGGAACGCCATCCACATGACT	660
	Y T V E D I I L F W D D N G N A I H M T	
661	GAGGAGCTGCATATCCCTCAGTTCACTTTCCTGGGAAGGACGATTACTAGCAAGGAGGTG	720
	E E L H I P Q F T F L G R T I T S K E V	
721	TATTTCTACACAGGTTCCCTACATACGCCTGATACTGAAGTTCCAGGTTCCAGAGGGAAGTT	780
	Y F Y T G S Y I R L I L K F Q V Q R E V	
781	AACAGCTACCTTGTGCAAGTCTACTGGCCTACTGTCCTCACCACTATTACCTCTTGGATA	840
	N S Y L V Q V Y W P T V L T T I T S W I	

841 TCGTTTTGGATGAACTATGATTCCTCTGCAGCCAGGGTGACAATTGGCTTAACTTCAATG 900
S F W M N Y D S S A A R V T I G L T S M

901 CTCATCCTGACCACCATCGACTCACATCTGCGGGATAAGCTCCCCAACATTCCTGTATC 960
L I L T T I D S H L R D K L P N I S C I

961 AAGGCCATTGATATCTATATCCTCGTGTGCTTGTCTTTGTGTTCTGTCCTTGCTGGAG 1020
K A I D I Y I L V C L F F V F L S L L E

1021 TATGTCTACATCAACTATCTTTTCTACAGTCGAGGACCTCGGCGCCAGCCTAGGCGACAC 1080
Y V Y I N Y L F Y S R G P R R Q P R R H

1081 AGGAGACCCCGAAGAGTCATTGCCCGCTACCGCTACCAGCAAGTGGTGGTAGGAAACGTG 1140
R R P R R V I A R Y R Y Q Q V V V G N V

1141 CAGGATGGCCTGATTAACGTGGAAGACGGAGTCAGCTCTCTCCCCATCACCCAGCGCAG 1200
Q D G L I N V E D G V S S L P I T P A Q

1201 GCCCCCCTGGCAAGCCCGGAAAGCCTCGTTCCTTTGACGTCCACCTCCGAGCAGGCCCCAG 1260
A P L A S P E S L G S L T S T S E Q A Q

1261 CTGGCCACCTCGGAAAGCCTCAGCCCACTCACTTCTCTCTCAGGCCAGGCCCCCCTGGCC 1320
L A T S E S L S P L T S L S G Q A P L A

1321 ACTGGAGAAAGCCTGAGCGATCTCCCCTCCACCTCAGAGCAGGCCCGGCACAGCTATGGT 1380
T G E S L S D L P S T S E Q A R H S Y G

1381 GTTCGCTTTAATGGTTTCCAGGCTGATGACAGTATTTTTCTACCGAAATCCGCAACCGT 1440
V R F N G F Q A D D S I F P T E I R N R

1441 GTCGAAGCCCATGGCCATGGTGTACCCATGACCATGAAGATTCCAATGAGAGCTTGAGC 1500
V E A H G H G V T H D H E D S N E S L S

1501 TCGGATGAGCGCCATGGCCATGGCCCCAGTGGGAAGCCCATGCTTCACCATGGCGAGAAG 1560
S D E R H G H G P S G K P M L H H G E K

1561 GGTGTGCAAGAAGCAGGCTGGGACCTTGATGACAACAATGACAAGAGCGACTGCCTTGCC 1620
G V Q E A G W D L D D N N D K S D C L A

1621 ATTAAGGAGCAATTCAAGTGTGATACTAACAGTACCTGGGGCCTTAATGATGATGAGCTC 1680
I K E Q F K C D T N S T W G L N D D E L

1681 ATGGCCCATGGCCAAGAGAAGGACAGTAGCTCAGAGTCTGAGGATAGTTGCCCCCAAGC 1740
M A H G Q E K D S S S E S E D S C P P S

WO 98/49293

PCT/GB98/01206

1741 CCTGGGTGCTCCTTCACTGAAGGGTTCTCCTTCGATCTCTTTAATCCTGACTACGTCCCA 1800
P G C S F T E G F S F D L F N P D Y V P

1801 AAGGTCGACAAGTGGTCCCGGTTCCCTCTTCCCTCTGGCCTTTGGGTTGTTCAACATTGTT 1860
K V D K W S R F L F P L A F G L F N I V

1861 TACTGGGTATAACCATATGTATTAG 1884
Y W V Y H M Y *

FIGURE 3