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(57) Abstract

Described is a novel subtype of metabotropic glutamate receptors, nucleic acid molecules encoding such receptors, vectors comprising the described nucleic acid molecules, as well as antibodies directed against said receptor. Furthermore, nucleic acid probes are described which specifically hybridize with the nucleic acid molecules, pharmaceutical and diagnostic compositions, transgenic non–human animals as well as methods to identify agonists and antagonists of the described receptor.

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NUCLEIC ACID MOLECULES ENCODING A GLUTAMATE RECEPTOR

The present invention relates to a glutamate receptor, to nucleic acid molecules encoding such a receptor, to vectors comprising the described nucleic acid molecules, host cells comprising such nucleic acid molecules or vectors as well as to antibodies directed to the receptor. Furthermore, the present invention relates to nucleic acid probes specifically hybridizing to the described nucleic acid molecules, to pharmaceutical and diagnostic compositions and to nonhuman transgenic animals. The present invention also relates to methods for identifying agonists and antagonists of the described glutamate receptor.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including any manufacturer's specifications, instructions, etc.) are hereby incorporated herein by reference; however, there is no admission that any document cited is indeed prior art as to the present invention.

L-glutamate is recognized as the major excitatory neurotransmitter in the central nervous system (CNS). The action of L-glutamate is mediated by both ionotropic receptors which form ion channels and which mediate fast excitatory synaptic transmission and metabotropic receptors (for review see Watkins, Trends Pharmacol. Sci. 11 (1990), 25-33; Schoepp, Trends Pharmacol. Sci. 14 (1993), 13-20). Metabotropic glutamate receptors (mGlu receptors) possess seven putative transmembrane spanning segments and can mediate at least some of their effects via G-proteins (guanine nucleotide binding proteins). They are functionally linked to intracellular effectors involved in several second messenger pathways and interact

directly or indirectly with a variety of membrane proteins such as ion channels (for review see Nakanishi, Neuron 13 (1994), 1031-1037; Nakanishi, Prog. Clin. Biol. Res. 390 (1994), 85-98; Nakanishi, Science 258 (1992), 597-603).

Metabotropic receptors have specific physiological functions such as in memory acquisition and learning, but they are also involved in pathological events. A variety of diseases are thought to result from excessive activation of mGlu receptors or from a defect in the cellular mechanisms that protect against the potential adverse consequences of physiological mGlu receptor activation. These disorders include epilepsy, focal and global ischaemia, pain, and neurodegenerative diseases as well as neuropsychiatric disturbances (for review see Knöpfel, J. Med. Chem. 38 (1995), 1417-1426; Knöpfel, Drug Discovery Today 1 (1996), 103-108).

The existence of mGlu receptors was first postulated in 1985 based on biochemical studies demonstrating that L-glutamine stimulates inositol triphosphate (IP3) accumulation in the central nervous system (Sladeczek, Nature 317 (1985), 717-719; Nicoletti, J. Neurochem. 46 (1986), 40-46). A cDNA encoding the rat mGluR1a was cloned by Masu (Nature 349 (1991), 760-765) and Houamed (Science 252 (1991), 1318-1321). Metabotropic glutamate receptors (mGluR) include a number of subgroups.

Molecular cloning has identified eight different subtypes of mGlu receptors each of which is encoded by a unique gene. The signal transduction mechanisms and agonist selectivities of each of these subtypes have been studied after DNA transfection of individual receptor cDNA clones (Knöpfel, (1995) loc. cit.). All of them are found to be expressed in various brain areas and mGluR6 is also active in the retina. Based on sequence homology, cellular effector pathways and response to receptor agonists, the mGlu receptors are subdivided into three groups.

In brain cells each mGluR subtype mediates characteristic and often cell type specific effects (Knöpfel, (1995) loc. cit.). Not all of the effects associated with the activity of mGlu receptors could be ascribed to known subtypes, indicating that there are not yet known subtypes or even not yet known groups.

Thus, in order to be able to more completely establish the functions of specific mGluRs both in normal cell physiology and in pathological conditions, it will be necessary to identify new mGlu receptor subtypes.

Furthermore, the cloning and pharmacological characterization of mGlu receptors is a key component for the further development of efficient drug screening assays and will be necessary to enable the discovery and further development of mGlu receptor ligands which selectively interact, and particularly bind to, specific mGlu receptor subtypes. A full set of cloned mGlu receptor subtypes is necessary to help to enhance any drug discovery method based on recombinant receptors and aiming at subtype or group specific ligands.

Thus, the technical problem underlying the present invention is to provide novel metabtropic glutamate receptors and nucleic acid molecules encoding such receptors.

This technical problem is solved by the provision of the embodiments as defined in the claims.

Thus, the present invention relates to a metabotropic glutamate family receptor having the following characteristics:

- it is a metabotropic glutamate receptor;
- activation of the receptor leads to activation of an intracellular effector pathway via cAMP and also stimulates inositol triphosphate accumulation;
- it is activated by agonists of group II mGlu receptors and also activated by agonists of group I mGlu receptors; and
- it reacts with antibodies specifically directed against group II or III mGlu receptors;
- it does not react with antibodies specifically directed against group I mGlu receptors; and
- it has an apparent molecular weight in SDS page of about 140 kD.

Such a receptor is a novel subtype of a metabotropic glutamate receptor which differs in several aspects from the metabotropic glutamate receptors so far identified.

As mentioned above, the mGlu receptors are subdivided into three groups, namely groups I, II and III, based on sequence homology, cellular effector pathways and their response to agonists. In particular, group I mGlu receptors lead upon activation to an intracellular effector pathway via IP3 or Ca^{2+}. In contrast thereto, group II and III receptors lead upon activation to an intracellular effector pathway via an inhibitory G-protein ($G_{i/o}$) that reduces the production of cAMP. Furthermore, group I mGlu receptors respond to the binding of certain ligands (e.g. 3,5-dihydroxyphenylglycine or quisqualate), whereas group II and III receptors are not effected by these ligands. On the other hand, agonists for group II (e.g. L-CCGI; (2S,1' S, 2' S) 2 (carboxycyclopropyl)) or III (e.g. L-AP4; (S) 2 amino-4-phosphonobutryc acid) mGlu receptors do not activate group I mGlu receptors. The receptor molecules according to the invention have been classified as glutamate receptors since they are activated by glutamate. Furthermore, they are identified as metabotropic receptors due to their property to couple through typical G-protein mediated second messengers, e.g. cAMP. Furthermore, structural similarities could be demonstrated by Western Blot analysis and PCR technology.

However, the described mGlu receptor does not belong to any of the above-defined groups of mGlu receptors so far known.

In particular, activation of the mGlu receptor according to the invention leads to activation of an intracellular effector pathway via cAMP and also to a stimulation of inositol triphosphate accumulation. The characteristics for group II or group III mGlu receptors differ from those of the mGlu receptors of the present invention. Group II and III receptors are coupled through an inhibitory G-protein, which leads to the down-regulation of cAMP. Furthermore, the encoded mGlu receptors according to the invention show a different pharmacology towards specific ligands directed against group II mGlu receptors.

Furthermore, the mGlu receptors according to the invention react with antibodies directed against group II or III mGlu receptors but not with antibodies directed

against group I mGlu receptors. On the other hand, in Western blot experiments it turned out that the described mGlu receptors have an apparent molecular weight of about 140 kD. In contrast thereto, group II or III mGlu receptors normally have a molecular weight of about 80 to 90 kD.

Preferably, the mGlu receptors of the present invention are predominantly expressed in liver. They may, however, also be expressed although to a lower level in the brain and the kidney. Thus, the mGlu receptors described in the present invention belong to a novel, so far unidentified group of mGlu receptors. Furthermore, the present invention relates to nucleic acid molecules encoding the metabotropic glutamate receptor according to the invention. Such nucleic acid molecules can be isolated according to methods described in more detail in the accompanying examples. Preferably, the nucleic acid molecules encoding the novel mGlu receptor comprise the nucleotide sequence of the liver 2/dna shown in Figure 5; see also example 2.

The nucleic acid molecules encoding the novel mGlu receptor open up new possibilities for the treatment of diseases connected with an abnormality in mGlu receptor activity. For example, the treatment of disorders caused by excessive mGlu receptor activity with certain pharmaceutical compositions often has severe side effects in various organs, i.e. damages of the liver. These could be caused by interaction of the active ingredients of the pharmaceutical compositions with receptors in the liver. The present invention now provides mGlu receptors which are preferably predominantly expressed in the liver and which can be tested for their interaction with pharmaceutical compositions. This might, for example, allow to identify pharmaceutical compositions which lead to damaging side effects in the liver due to an interaction or not-interaction with the liver-specific mGlu receptor.

Furthermore, the nucleic acid molecules according to the present invention may comprise a polynucleotide strand the complement of which hybridizes under stringent conditions to one of the above-described strands and which encodes the described mGlu receptor. "Stringent conditions" are preferably conditions as

described in Sambrook (Molecular Cloning, A Laboratory Manual, 2nd edition (1989), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

Such hybridizing sequences show preferably an identity of at least 50 %, more preferably of at least 70 % and most preferably of at least 90 % on the nucleic acid level to the sequences described above. These hybridizing sequences encode a mGlu receptor having the characteristics of a mGlu receptor as described in the present invention. The molecules hybridizing to the nucleic acid molecules of the invention thus also comprise fragments, derivatives and allelic variants of the above-described nucleic acid molecules which encode a mGlu receptor as described in the present invention. In this regard, fragments are defined as parts of the nucleic acid molecules, which are long enough in order to encode the described mGlu receptor. The term derivatives means that the sequences of these hybridizing molecules differ from the sequences of the above-mentioned nucleic acid molecules at one or more positions and that they exhibit a high degree of homology to these sequences. Hereby, homology means a sequence identity of at least 50 %, in particular an identity of at least 60 %, preferably of more than 70 % and still more preferably a sequence identity of more than 90 %. The deviations occurring when comparing with the above-described nucleic acid molecules might have been caused by deletion, substitution, insertion or recombination.

Moreover, homology means that functional and/or structural equivalence exists between the respective nucleic acid molecules or the proteins they encode. The nucleic acid molecules, which are homologous to the above-described molecules and represent derivatives of these molecules, are generally variations of these molecules that constitute modifications which exert the same biological function. These variations may be naturally occurring variations, for example sequences derived from other organisms, or mutations, whereby these mutations may have occurred naturally or they may have been introduced by means of a specific mutagenesis. Moreover, the variations may be synthetically produced sequences. The allelic variants may be naturally occurring as well as synthetically produced variants or variants produced by recombinant DNA techniques.

The proteins encoded by the various variants of the nucleic acid molecules according to the invention exhibit certain common characteristics. Biological activity, molecular weight, immunological reactivity, conformation etc. may belong to these characteristics as well as physical properties such as the mobility in gel electrophoresis, chromatographic characteristics, sedimentation coefficients, solubility, spectroscopic properties, stability, pH-optimum, temperature-optimum etc. Biological activity may comprise, for example, ligand binding or triggering of a certain intracellular second messenger response.

Important characteristics of the mGlu receptor encoded by the nucleic acid molecules according to the invention are those already mentioned above, in particular, the following:

The nucleic acid molecules according to the invention which are endogenous in mammalian cells are preferably expressed predominantly in the liver. They are also expressed preferably to some extent in the brain and/or in the kidney and expression is low or undetectable in other tissues. This can be verified, e.g. by Northern blot analysis, by in situ-hybridization or by immunostaining of tissue sections with antibodies specific for the described mGlu receptor.

The full-length receptor encoded by a nucleic acid molecule according to the invention has in an SDS page an apparent molecular weight of about 140 kD.

The encoded mGlu receptor is activated by agonists which activate group II mGlu receptors but is also activated by agonists of group I mGlu receptors. Furthermore, activation of the encoded mGlu receptor leads to an increase in the intracellular cAMP level. This can be measured, for example, as described in Sortino (J. Neurosci. 8 (1996), 2407-2415). Also the stimulation of inositol triphosphate (IP_3) accumulation can be detected.

Moreover, the encoded mGlu receptor is recognized by antibodies against group II or III mGlu receptors but not by antibodies against group I mGlu receptors.

Nucleic acid molecules hybridizing to the molecules according to the invention may be isolated e.g. from genomic libraries or from cDNA libraries produced from tissue, preferably from liver tissue, using methods well-known in the art, as described, for

example, in Sambrook et al., 1989, Molecular Cloning , A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

The present invention also relates to nucleic acid molecules comprising a polynucleotide strand the coding sequence of which differs from a polynucleotide strand described above due to the degeneracy of the genetic code.

The nucleic acid molecules according to the invention can be single-stranded or double-stranded. Thus, they may either comprise the coding strand, the strand complementary to the coding strand or both strands.

Furthermore, the present invention also relates to nucleic acid molecules containing a fragment of a nucleic acid molecule according to the invention as described above that encodes at least one functional domain of the mGlu receptor. The term "functional domain" means a domain of the receptor protein that is necessary to fulfill at least one of the functions of the receptor required for its biological activity, for example, ligand binding or signal transduction. In particular, the receptor encoded by the nucleic acid molecules according to the invention is supposed to comprise a transmembrane domain, preferably of seven putative transmembrane spanning segments, an extracellular domain and an intracellular domain. The extracellular domain is responsible for ligand binding, i.e. for glutamate binding, whereas the intracellular domain is responsible for triggering the intracellular second messenger response.

The nucleic acid molecules encoding at least one functional domain of the mGlu receptor also encompass such molecules which encode fusion proteins which contain at least one functional domain of the mGlu receptor and other sequences from other polypeptides. Other polypeptides may be, for instance, other membrane-located receptors. It is, for example, possible, to replace the transmembrane region of the mGlu receptor according to the invention by the transmembrane region of another membrane-located receptor. It is also possible to replace the extracellular domain of the mGlu receptor by the extracellular domain of another receptor or to

fuse the extracellular domain of the mGlu receptor to portions of other receptor molecules. Furthermore, the intracellular domain of the mGlu receptor can be fused to portions of other receptor proteins, e.g. to a transmembrane and extracellular domain. Thus, the nucleic acid molecules according to the invention also encompass those which encode chimeric polypeptides comprising at least one functional domain of a mGlu receptor described in the present invention.

In particular, the nucleic acid molecules according to the invention encompass those molecules which encode at least one functional domain of the mGlu receptor described in the present invention in combination with (a) domain(s) of other glutamate receptors. Such fusion proteins can be particularly useful for changing the coupling to G-proteins and/or improving the sensitivity of a functional assay. It is, for example, possible to exchange the intracellular domain of the mGlu receptor against the intracellular domain of a receptor which activates the phospholipase C/Calcium signaling. An example for such a receptor is the receptor mGluR5 (Minakami, J. Neurochem. (1995), 1536-1542). Particularly suitable for such an exchange is the second intracellular loop. In this way it is possible to analyze the interaction of a test compound with a ligand binding domain of the polypeptide of the invention using an assay for calcium ions.

The described chimeric receptor molecules cannot only be obtained by expressing corresponding nucleic acid molecules but also by crosslinking domains with agents known in the art.

The nucleic acid molecules according to the invention can be DNA or RNA molecules. In the case they are DNA molecules, they can be cDNA molecules or genomic DNA molecules. The nucleic acid molecules according to the invention can be isolated from natural sources, prepared by recombinant DNA techniques or chemically synthesized. Furthermore, they can be modified. For example, they can be labeled with a molecule or substance allowing their detection, with a toxic molecule or by the incorporation of nucleotide analogs.

The nucleic acid molecules according to the invention can be used, for instance, for diagnostic or therapeutic purposes. In connection with therapeutic purposes it is

possible to use the nucleic acid molecules in gene therapy approaches in order to treat diseases caused by an excessive activity or by an activity of the mGlu receptor according to the invention which is too low.

Furthermore, the present invention relates to a vector comprising a nucleic acid molecule according to the invention. Suitable vectors are, for example, those conventionally used in cloning techniques, i.e. plasmids, phages, phagemids, viruses, cosmids and the like.

In a preferred embodiment the nucleic acid molecule contained in such a vector is linked to regulatory elements allowing expression of the nucleic acid molecules in prokaryotic or eukaryotic host cells. Such regulatory elements are, for example, promoter sequences as well as enhancer sequences. In one embodiment of the present invention the regulatory elements allow for an inducible expression. For the expression in prokaryotic cells a multitude of promoters has been describe including, for example, the lac promoter or the trp promoter. Promoters suitable for expression in eukaryotic cells, in particular in mammalian cells, are, for instance, viral promoters such as the early and late SV40 promoter, the HSV thymikine kinase promoter, the CMV immediate early promoter, the mouse metallothionein-I promoter or the LTRs from retroviruses. The nucleic acid molecule can further be linked to a transcription termination signal (polyA-signal) if this is required.
If the nucleic acid molecule does not encode the complete mGlu receptor but only a fragment or domain thereof, it may also be linked to DNA sequences encoding peptide sequences which ensure secretion of the encoded protein. Such sequences are well known to the person skilled in the art.

The present invention also relates to a host cell genetically modified with a nucleic acid molecule according to the invention or with a vector comprising such a nucleic acid molecule. The term "genetically modified" means that the host cell comprises in addition to its natural genome a nucleic acid molecule or vector according to the invention which was introduced into the cell or into one of its predecessors. The

nucleic acid molecule or vector may be present in the genetically modified host cell either as an independent molecule outside the genome, preferably as a molecule which is capable of replication, or it may be stably integrated into the genome of the host cell.

The host cell according to the present invention may be any prokaryotic or eukaryotic cell. Suitable prokaryotic cells are those generally used for cloning, like E. coli or Bacillus subtilis. Eukaryotic cells comprise, for example, fungal or animal cells. Examples for suitable fungal cells are yeast cells, preferably those of the genus Saccharomyces and most preferably those of the species Saccharomyces cerevisiae. Suitable animal cells are, for instance, insect cells, vertebrate cells, preferably mammalian cells, and most preferably non-neuronal cells, such as, e.g. CHO or HEK293 cells.

The present invention also relates to a process for the preparation of a polypeptide encoded by a nucleic acid molecule according to the invention which comprises the steps of

(a) culturing a host cell according to the invention under conditions allowing expression of the polypeptide; and

(b) recovering the produced polypeptide from the culture, the cells or the culture medium.

Furthermore, the present invention relates to a polypeptide obtainable by the above-described process.

Such a polypeptide may be a polypeptide as encoded by the nucleic acid molecule according to the invention which only comprise naturally occurring amino acid residues, but it may also be a polypeptide containing modifications. These include covalent derivatives, such as aliphatic esters or amides of a carboxyl group, O-acetyl derivatives of hydroxyl containing residues and N-acyl derivatives of amino group containing residues. Such derivatives can be prepared by linkage to reactable groups which are present in the side chains of amino acid residues and at the N- and C-terminus of the protein. Furthermore, the polypeptide can be

radiolabeled or labeled with a detectable group, such as a covalently bound rare earth chelate, or conjugated to a fluorescent moiety. The polypeptide of the present invention can be, for example, the product of expression of a nucleotide sequence encoding such a protein, a product of chemical modification or can be purified from natural sources. Furthermore, it can be the product of covalent linkage of polypeptide domains.

The polypeptides according to the present invention may be complete receptor molecules or may represent domains of such a receptor, for example, a soluble fragment. Furthermore, they can be in the form of covalent or aggregative conjugates with other chemical moieties.
The polypeptides of the present invention can be used, for example, for therapeutic purposes, i.e. for the treatment of focal or global ischemia, Alzheimer's disease, epilepsy, pathological anxiety, pain and/or neurodegenerative diseases. Furthermore, the polypeptides according to the invention can be used as immunogens, for the generation of antibodies, in drug screening assays, as reagents in diagnostic assays, such as immunoassays, and in purification methods, such as affinity purification of a ligand binding to the polypeptide.

The present invention furthermore relates to an antibody which specifically binds to a polypeptide according to the present invention. Methods for the preparation of polyclonal or monoclonal antibodies as well as for the preparation of fragments thereof are well-known in the art. Such antibodies can be used for diagnostic purposes or for therapeutic purposes, i.e. for the treatment of focal or global ischemia, Alzheimer's disease, epilepsy, pathological anxiety, pain and/or neurodegenerative disorders.

Furthermore, the present invention relates to a nucleic acid probe of at least 15 nucleotides capable of specifically hybridizing with the nucleic acid molecule according to the invention. The term "specifically" in this regard means that the nucleic acid probe does not cross-hybridize under stringent conditions with nucleic

acid molecules encoding other known proteins. Such nucleic acid probes can be used, for example, as hybridization probes to identify and isolate related sequences, e.g. from cDNA or genomic libraries. Furthermore, they can be used as primers for PCR or other amplification reactions as well as for the detection of expression of a nucleic acid molecule according to the invention. This can be done, for example, by a method which comprises obtaining mRNA from the cells to be investigated, contacting the so obtained mRNA with the nucleic acid probe and detecting hybridization of the probe with the mRNA. Furthermore, these nucleic acid probes can be used in diagnostic methods, e.g. for the detection of a mutation in a gene encoding a mGlu receptor according to the invention. These nucleic acid probes can also be used for therapeutic purposes. The nucleic acid probe of the invention can be any polynucleotide, in particular DNA or RNA.
The nucleic acid probe according to the invention can also comprise chemical analogues of nucleotides and can be modified or labeled with a substance allowing its detection.

In a preferred embodiment the nucleic acid probe of the present invention is an RNA molecule which is capable of specifically hybridizing to a transcript of a nucleic acid molecule according to the invention, i.e. an antisense-RNA.

The present invention is also directed to antagonists/inhibitors which are specific for the mGlu receptor of the present invention. Antagonists/inhibitors are understood as molecules or compounds which negatively affect the biological activity and/or functionality of the mGlu receptor. This can be measured, for example by determining whether the second messenger response induced by the agonist and mediated by the receptor decreases. An example for such an antagonist/inhibitor is an antibody which binds specifically to the extracellular domain of the polypeptide of the present invention and thereby prevents the binding of ligands.
Another example for an antagonist/inhibitor is a chemical compound which specifically binds to a domain of the polypeptide of the invention and thereby blocks

the activation of the receptor, i.e. the triggering of an intracellular second messenger response.
The antagonist/inhibitor according to the present invention may be used for therapeutic purposes, e.g. for the treatment of focal or global ischemia, Alzheimer's disease, epilepsy, pathological anxiety, pain and/or a neurodegenerative disease.

Furthermore, the present invention relates to agonists of the polypeptide of the present invention. Agonists are understood as compounds or molecules capable of specifically enhancing the biological activity or functionality of the protein according to the invention.
This can be measured, for example, by determining whether a second messenger response triggered by the receptor is enhanced or whether the activation by a known agonist is prevented.

The present invention also relates to a pharmaceutical composition comprising the receptor according to the invention, a nucleic acid molecule according to the invention, an antibody according to the invention, a nucleic acid probe according to the invention, an antagonist or an agonist according to the invention or a combination of any of these components.
Such a pharmaceutical composition may furthermore comprise a pharmaceutically acceptable carrier.

Furthermore, the present invention relates to a diagnostic composition or a kit comprising a nucleic acid molecule, a polypeptide, an antibody or a nucleic acid probe according to the invention or a combination of any of these components, and optionally suitable means for detection.

In another aspect the present invention relates to a method for producing a compound capable of binding to the polypeptide according to the invention comprising the steps of

(a) contacting the polypeptide of the present invention, cells expressing said polypeptide on their surface or a membrane preparation of such cells with a compound or with a mixture of compounds to be tested for binding;

(b) detecting the presence of a compound bound to the polypeptide; and optionally

(c) isolating the compound which has been found to bind from said mixture.

In a preferred embodiment of such a method the cells used in step (a) are genetically modified cells according to the invention which contain in their cell membrane a mGlu receptor of the present invention. Accordingly, the membrane preparation used in step (a) is preferably derived from such cells.

The present invention also relates to the compound obtainable by the above-described method as well as to a pharmaceutical composition comprising such a compound, optionally further comprising a pharmaceutically acceptable carrier.

Furthermore, the present invention relates to a method for producing a ligand capable of binding to the polypeptide of the present invention and to functionally activate receptor activity comprising the steps of

(a) contacting a cell expressing on its surface an mGlu receptor according to the invention with a substance to be tested under conditions permitting the activation of a functional response; and

(b) detecting the presence of a functional response thereby determining whether the substance to be tested activates the mGlu receptor.

The present invention also relates to a method for producing a ligand capable of preventing activation of the mGlu receptor of the present invention comprising the steps of

(a) contacting a cell expressing on its surface an mGlu receptor according to the present invention with a substance to be tested and with a ligand which normally activates the receptor under conditions permitting activation of the receptor; and

(b) detecting the blockage of the functional response, thereby determining whether the substance to be tested prevents the activation of the receptor.

In one embodiment of the present invention the substance to be tested in the above-described methods is contained in a mixture of compounds and steps (a) and (b) are repeated after having further purified the bound substance from the mixture until the substance which binds to the receptor is obtained in pure form. The purification of the substance from the mixture before each repetition of steps (a) and (b) may be achieved e.g. by affinity chromatography on a column containing the receptor polypeptide or a part of it.

In a preferred embodiment of the above-described methods according to the present invention, the cells used are genetically modified cells according to the invention having in their cell membrane the mGlu receptor according to the invention.

In a further preferred embodiment the functional response detected in the methods is the increase in the intracellular cAMP level.

The present invention also relates to a ligand obtainable by the above-described methods as well as to a pharmaceutical composition comprising such a ligand and optionally further comprising a pharmaceutically acceptable carrier.

Furthermore, the present invention relates to a method for diagnosing a predisposition to a disorder associated with the expression of a nucleic acid molecule according to the invention which comprises the steps of:

(a) obtaining DNA from a sample of a subject suffering from the disorder;

(b) performing a restriction digest of the DNA with a panel of restriction enzymes;

(c) electrophoretically separating the resulting DNA fragments on a sizing gel;

(d) contacting the resulting gel with the nucleic acid probe of claim 13 labeled with a detectable marker;

(e) detecting labeled bands to create a unique band pattern specific to the DNA of subjects suffering from the disorder;

(f) preparing DNA obtained for diagnosis by steps (a) to (e); and

(g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) and the DNA obtained for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

Finally, the present invention relates to a transgenic non-human animal, preferably a mammal, genetically modified with a nucleic acid molecule according to the invention. Such a transgenic non-human animal can be prepared according to methods well-known in the art, such as those described, for example, in Nagy (Proc.Natl.Acad. Sci. USA 90 (1993), 8424-8428), in Gene Targeting, A technical Approach, Joyner (ed.), Oxford University Press and in Conquet (Neuropharmacology 34 (1995), 835-870).

The transgenic non-human animal may be genetically modified with a nucleic acid molecule according to the invention so as to express on all or on certain cells or cell types an mGlu receptor according to the invention. Preferably, the nucleic acid molecule according to the invention used for the genetic modification of the animal is placed under the control of an inducible promoter allowing induction of transcription, for example, by an exogenous stimulus, at a certain time or in a certain tissue. Such genetically modified non-human animal can be used, for example, to determine the effects of expressing varying levels of the mGlu receptor of the present invention.

Furthermore, the transgenic non-human animal can be genetically modified with a nucleic acid molecule according to the invention which has been modified so as to encode a mutated version of the mGlu receptor incapable of normal receptor activity.

Moreover, the transgenic non-human animal according to the invention can be genetically modified so as to show reduced expression or be devoid of any expression of nucleic acid molecules according to the invention. One possibility to generate such animal is the disruption of endogenous sequences encoding the mGlu receptor according to the invention. Methods for the preparation of such "knock out" animals are known in the art. Another possibility to generate such transgenic non-human animal is the expression of an antisense-RNA according to the invention.

Figure 1 shows the selective pharmacological effects of specific ligands of group II and III mGlu receptors in the liver.

Forskolin, a specific modulator of the adenylate cyclase, leads together with specific agonists for metabotropic receptors to an acute activation of cAMP in primary cells derived from the rat liver.

Figure 2 shows the selective pharmacological effects of specific ligands of group II and III mGlu receptors in the liver of neonatal rats.

Also in this case, forskolin leads together with specific agonists for metabotropic receptors to an acute activation of cAMP in primary cells derived from the rat liver.

Figure 3 shows the effects of ACPD on human HepG2 liver cells. Forskolin, a specific modulator of the adenylate cyclase, leads together with the specific agonist ACPD for class I and II metabotropic receptors to an acute activation of cAMP in human HepG2 liver cells.

Figure 4 shows the results of a Western Blot analysis. Different kinds of specific antibodies were used. These were directed against mGluR1(1), mGluR2 & 3 (2/3), mGluR3(3) and mGluR5(5). Extracts of brain (CTX) and of

liver tissues (L) were studied. A positive binding could be achieved by the group II antibodies.

Figure 5 shows a comparison of the partial mRNA sequence deposited with the Genebank Database under accession number N90493 with the nucleotide sequence of the metabotropic glutamate receptor 2.

The following examples further illustrate the present invention.

Example 1

Identification of a novel mGlu receptor activity

Various studies led to the identification of a new type of a metabotropic receptor in the liver of rats and in human cell lines. It could be shown by functional and pharmacological analysis that the expression of a novel G protein coupled mGluR is indicating a different metabotropic receptor class-I and group II pharmacology. Group I (quisqualate) and group II agonists (4C3HPG) were able to stimulate the new receptor subtype. In contrast to typical class II mGluRs the second messengers could be identified as cAMP that is been stimulated through the G protein (Gq). Immunostaining confirmed the group-II mGluR subtype, however the size of the labeled protein was about 140kd which is significantly larger than that of typical class II mGluRs detected in the brain. The novel mGlu receptor in the liver shares properties of group-I and -II mGlu receptors.
In particular, the following experiments led to the identification of a new type of a mGlu receptor:

Measurement of cAMP Formation. Rat liver cells and the human HepG2 liver cell line at 80-90% confluence were prepare and washed in oxygenated Krebs-Heneleit buffer and incubated at 37°C for 15min. Forskolin was added 5 min after all compounds. Cells were incubated with isobutyl methylxanthine (IBMX), when used, for 10 min prior to addition of agonists. When antagonists were used, a preincubation of 5 min was always carried out. Incubation was stopped by removal of the buffer and addition of ice-cold 0.4 M perchloric acid. Samples were then equilibrated by addition of 2 N K_2CO_3 and processed for measurement of cAMP content. The level of cAMP was quantitated by radioimmunoassay. The results are shown in Figure 1. It is evident from this figure that forskolin, a specific modulator of the adenylate cyclase leads togehter with specific agonists for metabotropic

receptors to an acute activation of cAMP in primary cells derived from the rat liver. Similar results were obtained when primary cells derived from the liver of neonatal rats were used (see Figure 2).

Determination of specific metabolic activity using the cytosensor. The cytosensor microphysiometer (McConnell, 1995) was used to evaluate for the functional and pharmacological expression of the identified liver mGlu receptor in the human HepG2 cell lines. Cells were plated at a density of 300.000 cells and stimulated using ACPD (100µM) and Forskolin (10µM) in a perfusion system. The results of this experiment are shown in Figure 3. As can be seen from this figure, in this case, forskolin leads together with the specific agonist of group I and II metabotropic receptors to an increase of more than 100% versus control cells.

Immunocytochemistry. In Western Blot analysis a membrane extract of primary rat liver cells was stained using an antibody that is recognizing an epitope present in the carboxyterminal end of group-II mGlu receptors. Those amino acid sequences were: VPTVCNGREVVDSTTSSL. The primary antibodies were diluted 1:1000 in NETG buffer, and the secondary antibody (peroxidase-coupled goat anti-rabbit; BioRAD) was diluted 1:1500. Specific bands were detected using the ECL Western blotting analysis system. The size of the labeled protein was 140kd. No such protein was detected in extracts from rat kidney, gut, lung, testis or spleen.

Pharmacological Data. Several specific agonists of mGluRs were used for analysis:

The group-I mGluR agonists 3,5-DHPG (3,5-dihydroxyphenylglycine) and quisqualate failed to stimulate phosphoinositide hydrolysis in the liver.

The group-II mGluR agonist 4C3HPG (4-carboxy-3-hydroxyphenylglycine) reduced norepinephrine stimulated cAMP formation in the liver of adult rats. Furthermore, both 4C3HPG and 1S,3R-ACPD potentiated the stimulation of cAMP by forskolin in the liver from newborn and reduced forskolin responses in adult animals as well as in the derived cell line.

Immunostaining & Immunoblotting. Membrane preparations of neonate and adult rat tissues showed a single band with an antibody recognizing an epitope present in the carboxyterminal end of group-II mGlu receptors. However, the size of the labeled protein was 140kD, which is significantly larger than that of mGlu2 or -3 receptors detected in the CNS (about 85kD). The results of this experiment are shown in Figure 4. No such protein was detected in other peripheral tissues of the rat. No immunolabeling was obtained with antibodies recognizing the remaining 20 amino acids of the carboxy-terminus end of group-I mGlu receptors (Jeffrey, Brain Research 25 (1996), 75-81). Immunostaining of cells confirmed group-II mGlu receptor homology.

Stimulation of polyphosphoinositide hydrolysis in primary cultures of rat hepatocytes. Adult rat hepatocytes were isolated by collagenase perfusion, plated into Falcon Pilmarla 24-multiwell dishes ($3x10^6$ cells/well), and grown up to 5 days under conditions supporting survival and liver specific phenotype (Ham's F12 medium containing 10% foetal calf serum, 0.2% albumin, 100 nM insuline and 100 nM dexamethasone) (according to the methods of Guguen-Guillouzo (Prog. Liver Dis. 8 (1986), 33-50) and Kimball (Am. J. Physiol. 268 (1995), 6-14); slighlty modified). Culture medium was changed every day. Cultures were incubated overnight with 1 µCi/ml of myo-2-[^{3}H]inositol (NEN-DuPont, sp. act. 16.5 Ci/nmol) for the labeling of inositol phospholipids. Cultures were then washed in Krebs-Henseleit buffer (equilibrated with O_2/CO_2 to pH 7.4) and incubated in the same buffer containing 10 mM Li^+. After 30 min of incubation in the presence of transmitter receptor agonists, the reaction was terminated by addition of methanol:water (1:1 v/v). [^{3}H]inositolmonophosphate ([^{3}H]InsP) was extracted, separated and detected as described previously (Nicoletti, J. Neurosci. 6 (1986), 1905-1911).

In cultures at 2 days *in vitro*, (1_S,3_R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD, 100 µM) and quisqualate (100 µM), increased [^{3}H]InsP formation by about 80%. The action of 1_S,3_R-ACPD was concentration-dependent with an apparent

EC_{50} value of 25 μM and a maximal stimulation at 100 μM. At 5 days *in vitro*, stimulation of polyphosphoinositide hydrolysis by both mGlu receptor agonists was maintained, albeit reduced by half, whereas (RS)-3,5-dihydroxyphenylglycine (DHGP) and (2_S, 3_S, 4_S)-α-(carboxycyclopropyl)glycine (L-CCG-I) were incative.

1_S,3_R-ACPD-stimulated polyphosphoinsitide hydrolysis was inhibited by the mixed mGlu receptor antagonist, α methyl 4-carboxyphenylglycine (MCPG, 500 μM) (Table 1).

Table 1 - Stimulation of polyphosphoinsositide hydrolysis by mGlu receptor agonists in cultured hepatocytes at 2 or 5 days *in vitro*.

	[^{3}H]InsP formation (d.p. m./mg protein)
<u>2 days *in vitro*</u>	
Basal	6075 ± 202
1_S,3_R-ACPD, 100 μM	11325 ± 150[a]
Quisqualate, 100 μM	10800 ± 675[a]
<u>5 days *in vitro*</u>	
Basal	4162 ± 225
1_S,3_R-ACPD, 100 μM	5887 ± 350[a]
Quisqualate, 100 μM	5650 ± 120[a]
DHPG, 100 μM	3973 ± 360
L-CCG-1, 100 μM	3862 ± 187
MCPG, 500 μM	4312 ± 185
1_S,3_R-ACPD + MCPG	3900 ± 150[b]

Values are means ± S.E.M. of 4-8 determinations. $P<0.01$. If compared to the respective basal (a) or 1_S,3_R-ACPD (b) values (One-way analysis of variance +

Fisher's test to isolate the differences). MCPG has been applied to the cultures 1 min before $1_S,3_R$-ACPD. MCPG was inactive at concentrations of 100 µM (not shown).

These results indicate that cultured hepatocytes express functionally active mGlu receptors coupled to polyphosphoinositide hydrolysis. In recombinant cells, DHPG behaves as a pure agonist of $mGlu_1$ and $_5$ receptor; quisqualate activates $mGlu_1$ and $_5$ receptors with high potency, and mGlu3 receptors with lower potency; $1_S,3_R$-ACPD and L-CCG-I activate both group-I and -II mGlu receptors with low and high potency, respectively (Pin and Duvoisin, Neuropharmacology 34 (1995), 1-26). The lack of effect of DHPG and L-CCG-I suggests that the activation of polyphosphoinositide hydrolysis in cultured hepatocytes is not mediated by conventional $mGlu_1$ or $_5$ receptors and neither results from a functional synergism between group-I and -II mGlu receptor subtypes.

Example 2

Cloning of a cDNA Encoding the identified mGlu receptor

A cDNA library (Invitrogen) derived from human liver was subjected to amplification by PCR with the use of a set of specific primers. The synthesis of these primers was based on the sequences corresponding to the external aminoterminal part and to the sixth transmembrane segments of the current set of available metabotropic glutamate receptors. The primers were designed to amplify only mGluR specific sequences. In order to determine whether the new mGluR cDNA clone exists in the library, we prepared DNA from the plate lysates (Sambrook, Molecular Cloning: A Laboratory Manual; 2nd Ed., (1989), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.) of the human liver library was prepared and PCR amplification with the cDNA as a template was performed. Oligonucleotides corresponding to the nucleotide positions 172-198 (5-GluR; SEQ ID NO: 1) and 464-489 (3-GluR; SEQ

ID NO: 2) of the human mGluR were used as primers. The positions refer to the human metabotropic glutamate receptor type II (GLUR2), Accession No. L 35318. These primers allowed binding to all three different classes of metabotropic glutamate receptors, which could be shown by multiple alignment. The nucleotide sequence of the PCR-amplified fragment revealed that a PCR product of 300 bp was amplified.

A parallel approach was taken by the use of a specific 3`-prime oligonucleotide (3-GluR2; SEQ ID NO: 4) that is designed to bind at a region of the cDNA at the positions 2587-2616. This primer was used in combination with a primer having the sequence as shown in SEQ ID No. 3. This primer is an oligonucleotide that binds at positions 2290-2320.

Furthermore, three primers were used for amplification that bind at positions 646-666 (Primer No. 5), 1952-1971 (Primer No. 6) and 2290-2310 (Primer No. 7), respectively. These can be used in the following combinations:

a) Primer No. 5 and Primer No. 7;

b) Primer No. 6 and Primer No. 7.

Primer No. 5 binds in the transmembrane domain III overlapping the cytoplasmic portion. Primers No. 6 and 7 bind in a region encoding the cytoplasmic portion of the mGlu receptors. Using these primer pairs, it was possible to obtain amplification products of 1600 bp and 360 bp, respectively.

Primer sequences:

Primer 5-GluR: 5`-GGC ATT CAG AGA GTG GAG GCC ATG CT -3`

(SEQ ID No: 01) T C A C C C A G T

G C G

Primer 3-GluR: 5`-ATC TGA GGT ATG TTG AAA AGC TGG A -3`

(SEQ ID No: 02) T G C GA G G CTC

G C T A

Primer 5-GluR1: 5`-TTC ACC ATG TAC ACC ACC TGC ATC ATC TGG -3`
(SEQ ID No: 03) A G T T A

Primer 3-GluR2: 5`-AGC GAT GAC GAA GAC GAC TCC ACC ACC TC -3`
SEQ ID No: 04) G C G GG T

Primer 5: 5'-TAT GGI GA(AG) AII GGI (AG)T (GT) GA -3'

Primer 6: 5'-TIA CIA A(AG)A C(AC)A A(CT)C CIA T -3'

Primer 7: 5'-CAC GT(GC) GTG TAC AT(AGT) GTG AA -3'

All amplified fragments are cloned into vectors and are used for further analysis. For this purpose, full length cDNA clones of hmGluR5 and hmGluR2 are used for hybridization to select for positive cDNA fragments derived from human liver. Positive clones can then be used to obtain the complete cDNA from the originally used liver cDNA library.

A parallel approach is taken by the use of a short DNA sequence from the glutamate binding site of the human metabotropic glutamate receptor type 2 (GLUR2), accession no. L 35318 which was identified with NCBI BLAST Search; Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David Lipman (1990). Basic local alignment search tool. J. Mol. Biol. 215, 403-410. For this virtual screening, nucleotide sequence 5'-AGTGATGTCTCC ATCCAGGTGGCCAACCTCTTGAGGCTATTTCAGATCCCACAGATTAGCTAC-3' (SEQ ID NO. 8) which corresponds to nucleotides 435 to 497 of the human GLUR2

sequence was used. The screening of the Genebank Database revealed a human EST clone deposited under accession no. N90493; Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone 292835 5', mRNA sequence. This DNA sequence was designated liver2/dna and compared with the GLUR2 sequence; see Fig. 5. The regions that show significant homology are boxed. Based on these homologies, two new primers for PCR were designed. (Fig. 5: LIV-5': (bp 47-67 of liver2/dna: 5'-C(CT)(GC)ATG(GT)A(AT)CA(AG)(AC)CAC(AT)TCT-3' (SEQ ID NO. 9) and LIV-3': (bp 190-211 of liver2/dna: 5'-CT(GT)GT(AG)G(AG)(AG)(GC)(CT)G(AT)A(GC)CT(AT)A(AT)G-3' (SEQ ID NO. 10)). (The nucleotides in brackets indicate that the primers are degenerate). These primers were used to clone a cDNA fragment from rat liver tissue and the human HepG2 liver cell line. The following conditions and materials were used for PCR:

5 min 94°C

-1 min 94°C
-1 min 55; 60; 65°C; 35 cycles
-2 min 72°C

2 min 72°C

cycler: -RoboCycler 40; Stratagene.
PCR chemicals: Advantage GC-cDNA PCR kit (Clontech, K1907-1)
amount of template DNA: 250 ng
amount of primer: 100 ng

The PCR product thus obtained is 166 base pairs in length; see also Fig. 5. This fragment is then used for the screening of a liver cDNA library (Uni-ZAP XR library of Stratagene catalog no. # 937241). The titer of the library was adjusted such that about 8,000 single clones/15 cm dishes are obtained in the first screening. These clones were then transferred on nylon filters (Biodyne Transfer Membrane, PALL; BNNG 132). The DNA was cross-linked with UV at 2,000 Joule. Hybridization was performed with a radioactively labeled probe that was obtained via PCR of the 166 bp fragment with α^{32} P-dCTP (Amersham Pharmacia Biotech). The hybridization conditions are as follows:

Prehybridizing:

For at least 3 hrs in Rupperts solution (0.25 M Na_2HPO_4, pH 7.2; 7% SDS; 1% BSA; 1 mM EDTA; filtered through a 0.45 µm filter).

Hybridizing: over night in the same hybridization solution at 58-60°C.

Washing conditions: 3 x 20 min at 58-60°C in 30 mM Na_2HPO_4, 1% SDS.

Exposition of the filters: at -80°C over night to several days.

The filters that show positive signals are then re-screened and those clones that show reliably positive signals are then used to obtain the cDNA sequence and to obtain complete cDNA from the originally used liver cDNA library.

The proteins encoded by cDNAs contained in the clones identified by the methods as described above are then further characterized, for example with specific antibodies that recognize an epitope of conserved domains present in Glu receptors. Furthermore, the encoded protein can be analyzed with respect to the biological activity, for example as described in Example 1.

CLAIMS

1. A metabotropic glutamate family receptor having the following characteristics:
   - it is a metabotropic glutamate receptor;
   - activation of the receptor leads to activation of an intra cellular effector pathway via cAMP and also stimulates inositol triphosphate accumulation;
   - it is activated by agonists of group II mGlu receptors and also activated by agonists of group I mGlu receptors;
   - it reacts with antibodies directed specifically against group II or III mGlu receptors;
   - it does not react with antibodies specifically directed against group I mGlu receptors; and
   - it has an apparent molecular weight in SDS page of about 140 kD.

2. A nucleic acid molecule encoding the receptor of claim 1.

3. A nucleic acid molecule containing a fragment of the nucleic acid molecule of claim 2 that encodes at least one functional domain of said mGluR family receptor.

4. The nucleic acid molecule of claim 3, wherein said functional domain is the glutamate binding region, the transmembrane region or the intracellular region of said mGluR family receptor.

5. The nucleic acid molecule of any one of claims 2 to 4 which comprises the nucleotide sequence of the liver 2/dna shown in Figure 5.

6. The nucleic acid molecule of any one of claims 2 to 5 which is DNA or RNA.

7. A vector comprising a nucleic acid molecule of any one of claims 2 to 6.

8. The vector of claim 7, wherein the nucleic acid molecule is linked to regulatory elements allowing expression in prokaryotic or eukaryotic host cells.

9. A host cell genetically modified with the nucleic acid molecule of any one of claims 2 to 6 or with the vector of claim 7 or 8.

10. The host cell of claim 9 which is a mammalian cell.

11. A process for the preparation of a polypeptide encoded by the nucleic acid molecule of any one of claims 2 to 6 comprising the steps of

(a) culturing the host cell of claims 9 or 10 under conditions allowing expression of the polypeptide and

(b) recovering the produced polypeptide from the culture, the cells or the culture medium.

12. A polypeptide obtainable by the process of claim 11.

13. An antibody which specifically binds to the polypeptide of claim 1 or 12.

14. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with the nucleic acid molecule of any one of claims 2 to 6.

15. The nucleic acid probe of claim 14 wherein the nucleic acid molecule is an antisense-RNA capable of specifically binding to a transcript of the nucleic acid molecule of any one of claims 2 to 5.

16. An antagonist to the polypeptide of claim 1 or 12.

17. An agonist of the polypeptide of claim 1 or 12.

18. A pharmaceutical composition comprising
- the polypeptide of claim 1 or 12;
- the nucleic acid molecule of any one of claims 2 to 6;
- the antibody of claim 13;
- the nucleic acid probe of claim 14 or 15;
- the antagonist of claim 16; or the
- agonist of claim 17
- or a combination of any of these components.

19. A diagnostic composition comprising
- the polypeptide of claim 12;
- the nucleic acid molecule of any one of claims 2 to 6;
- the antibody of claim 13; and/or
- the nucleic acid probe of claim 14 or 15;
- or a combination of any of these components.

20. Use of the nucleic acid molecule of any one of claims 2 to 6, of the polypeptide of claim 1 or 12, of the antibody of claim 13, of the nucleic acid probe of claim 14, of an antagonist of claim 16 or of an agonist of claim 17 for the preparation of a pharmaceutical composition for the treatment of focal or global ischemia, Alzheimer's disease, epilepsy, pathological anxiety, pain and/or a neurodegenerative disease.

21. A method for producing a compound capable of binding to the polypeptide of claim 1 or 12 comprising the steps of

(a) contacting the polypeptide of claim 1 or 12, cells expressing said polypeptide on their surface or a membrane preparation of such cells with a compound or a mixture of compounds to be tested;

(b) detecting the presence of a compound bound to the polypeptide; and optionally

(c) isolating the compound which has been found to bind from said mixture.

22. A method for producing a ligand capable of binding to the polypeptide of claim 1 or 12 and to functionally activate receptor activity comprising the steps of

(a) contacting a cell expressing on its surface a polypeptide of claim 1 or 12 with a substance to be tested under conditions permitting the activation of a functional response; and

(b) detecting the presence of the functional response, thereby determining whether the substance to be tested activates the polypeptide of claim 1 or 12.

23. A method for producing a ligand capable of preventing activation of the polypeptide of claim 1 or 12 comprising the steps of

(a) contacting a cell expressing on its surface the polypeptide of claim 1 or 12 with a substance to be tested and with a ligand which normally activates the receptor, under conditions permitting activation of the receptor; and

(b) detecting the blockage of the functional response, thereby determining whether the substance to be tested prevents the activation of the receptor.

24. A compound or ligand obtainable by the method of any one of claims 21 to 23.

25. A pharmaceutical composition comprising the compound or ligand of claim 24 and optionally a pharmaceutically acceptable carrier.

26. A method for diagnosing a predisposition to a disorder associated with the expression of a nucleic acid molecule of claim 2 which comprises the steps of:

(a) obtaining DNA from a sample of a subject suffering from the disorder;

(b) performing a restriction digest of the DNA with a panel of restriction enzymes;

(c) electrophoretically separating the resulting DNA fragments on a sizing gel;

(d) contacting the resulting gel with the nucleic acid probe of claim 14 labeled with a detectable marker;

(e) detecting labeled bands to create a unique band pattern specific to the DNA of subjects suffering from the disorder;

(f) preparing DNA obtained for diagnosis by steps (a) to (e); and

(g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step (e) and the DNA obtained for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

27. A transgenic non-human mammal genetically modified with a nucleic acid molecule of any one of claims 2 to 6.

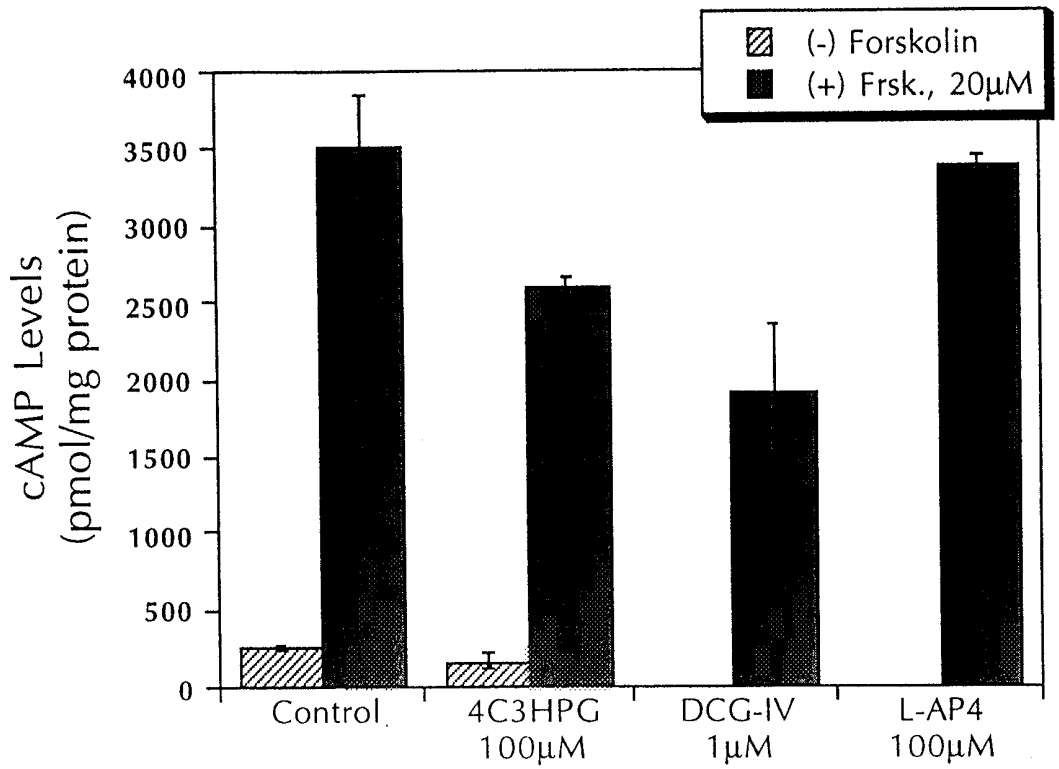

(-) Forskolin
(+) Frsk., 20µM
cAMP Levels
(pmol/mg protein)
4000
3500
3000
2500
2000
1500
1000
500
0
Control
4C3HPG
100µM
DCG-IV
1µM
L-AP4
100µM

Fig. 1

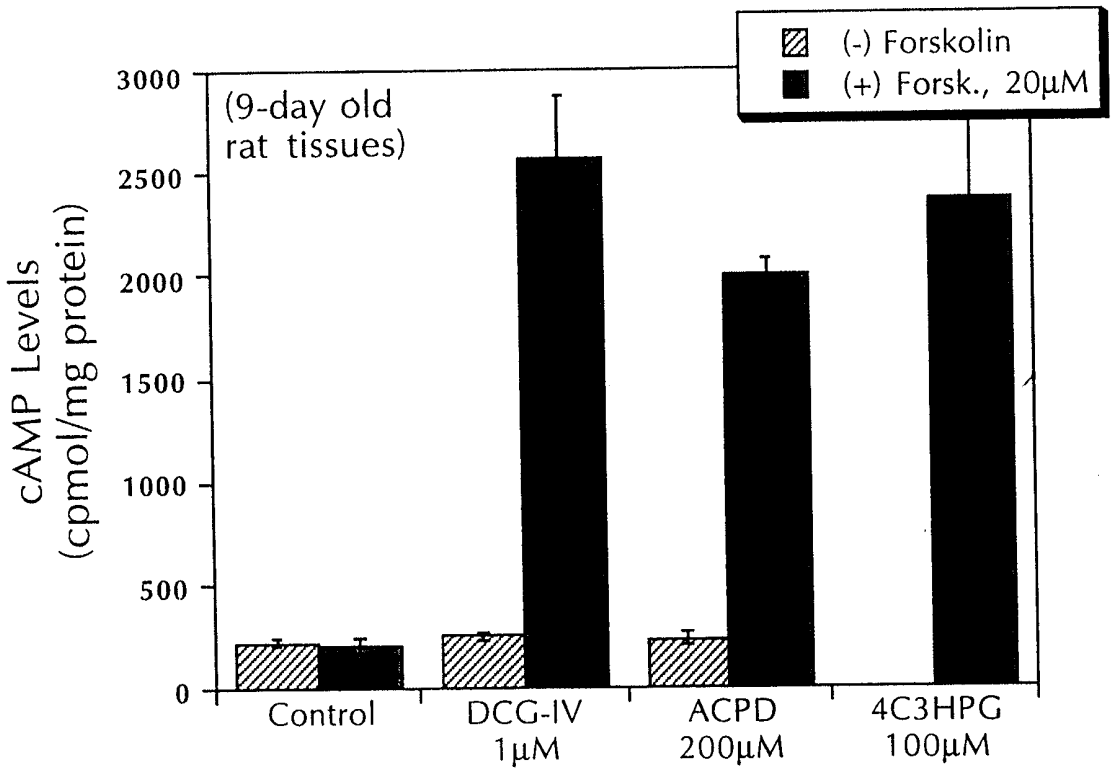

(-) Forskolin
(+) Forsk., 20µM
(9-day old
rat tissues)
cAMP Levels
(cpmol/mg protein)
3000
2500
2000
1500
1000
500
0
Control
DCG-IV
1µM
ACPD
200µM
4C3HPG
100µM

Fig. 2

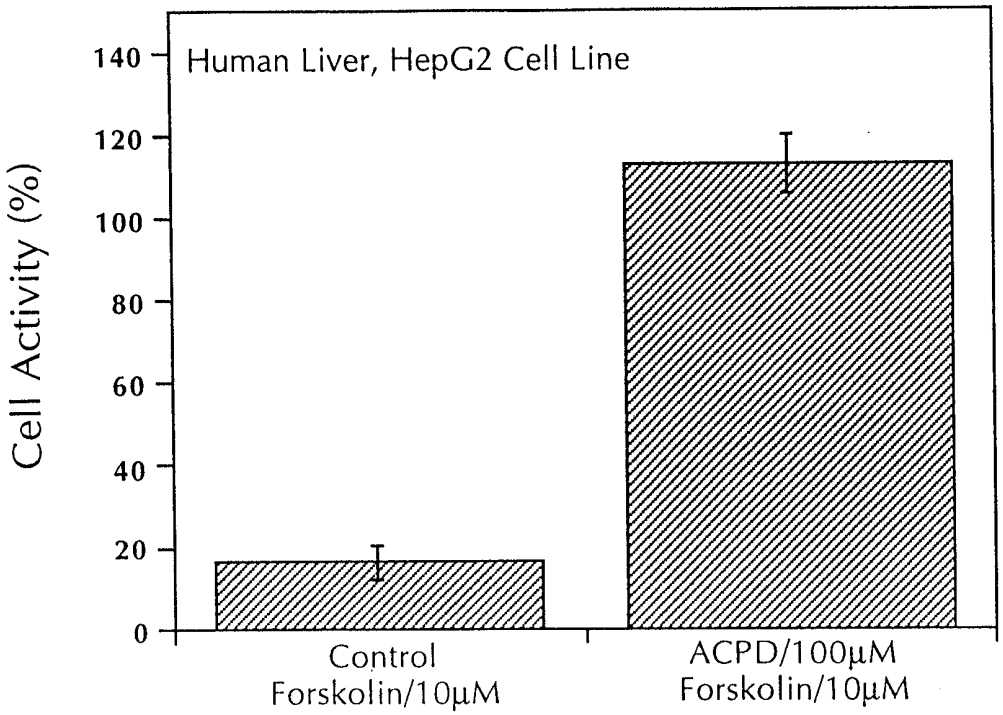

Human Liver, HepG2 Cell Line
Cell Activity (%)
140
120
100
80
60
40
20
0
Control
Forskolin/10µM
ACPD/100µM
Forskolin/10µM

Fig. 3

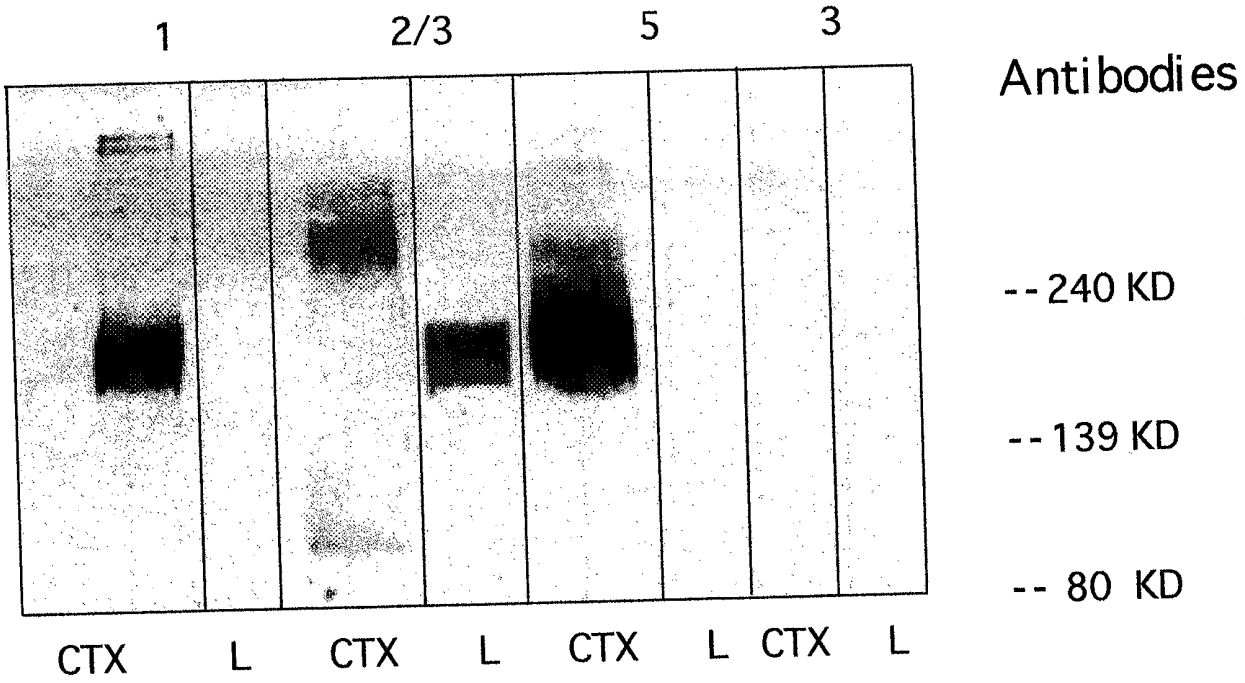

1
2/3
5
3
Antibodies
--240 KD
--139 KD
-- 80 KD
CTX
L
CTX
L
CTX
L
CTX
L

Fig. 4

liv/GluR2 align Formatted Alignment

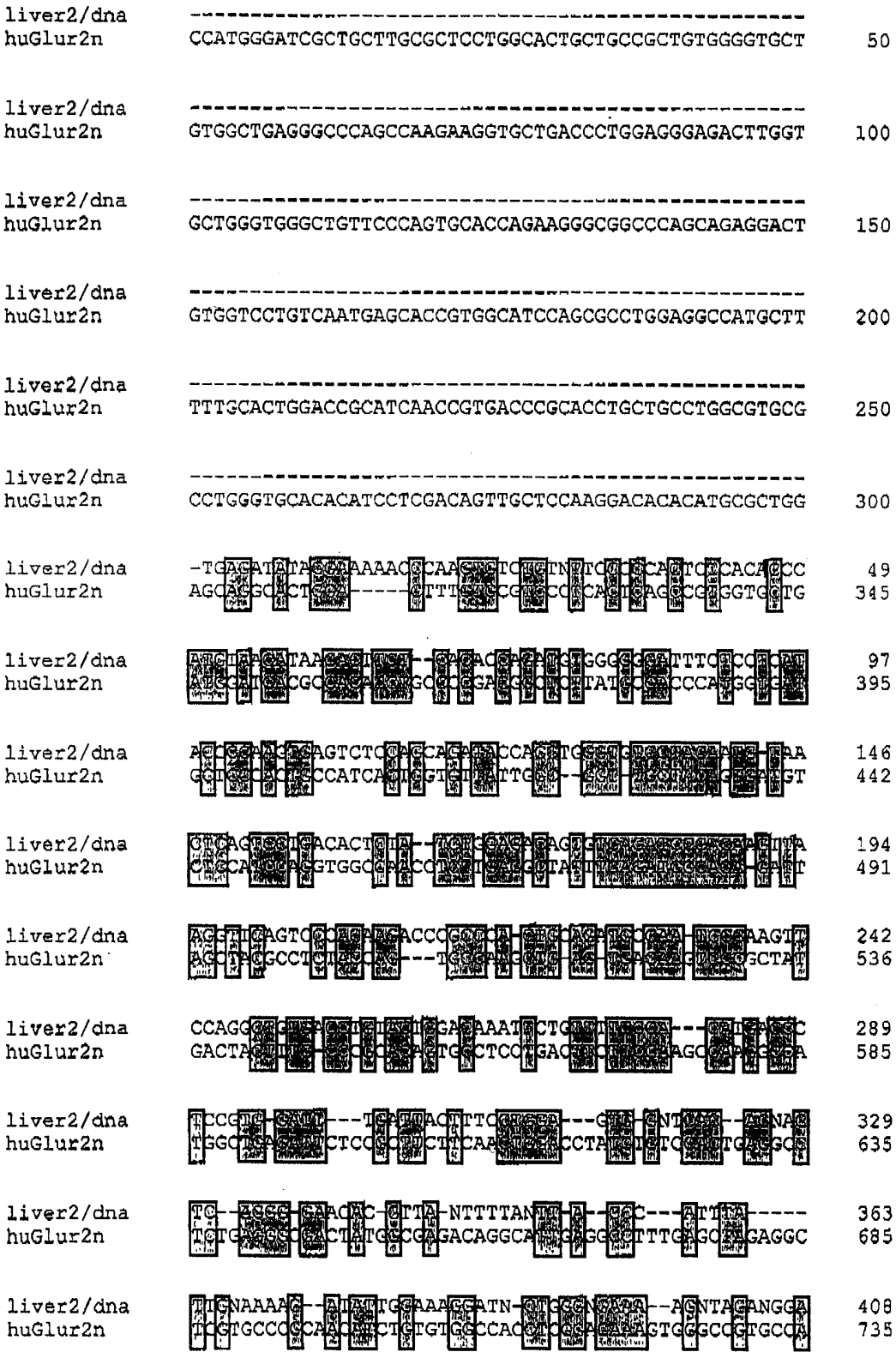


Fig. 5

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Dr. Rainer Metzger
(B) STREET: Kopfengartenstr. 7 B
(C) CITY: Landsberg
(E) COUNTRY: Germany
(F) POSTAL CODE (ZIP): 86899

(A) NAME: Dr. Ludwig Laxhuber
(B) STREET: Kientalstr. 28a
(C) CITY: Herrsching
(E) COUNTRY: Germany
(F) POSTAL CODE (ZIP): 82211

(ii) TITLE OF INVENTION: Nucleic acid molecules encoding a glutamate receptor

(iii) NUMBER OF SEQUENCES: 7

(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: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:3
(D) OTHER INFORMATION:/note= "May be C, T or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:6
(D) OTHER INFORMATION:/note= "May be T or C"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:9
(D) OTHER INFORMATION:/note= "May be G, A or C"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:10
(D) OTHER INFORMATION:/note= "May be A or C"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:12
(D) OTHER INFORMATION:/note= "May be A, C or G"

(ix) FEATURE:
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(D) OTHER INFORMATION:/note= "May be G or C"

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(A) NAME/KEY: modified_base
(B) LOCATION:18
(D) OTHER INFORMATION:/note= "May be G or A"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:21
(D) OTHER INFORMATION:/note= "May be C or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:25
(D) OTHER INFORMATION:/note= "May be C or T"

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

GGCATTCAGA GAGTGGAGGC CATGCT 26

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:6
(D) OTHER INFORMATION:/note= "May be A, T or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:9
(D) OTHER INFORMATION:/note= "May be T or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:12
(D) OTHER INFORMATION:/note= "May be G or C"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:14
(D) OTHER INFORMATION:/note= "May be T, G or C"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:15
(D) OTHER INFORMATION:/note= "May be G or A"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:18
(D) OTHER INFORMATION:/note= "May be A, G or T"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:21
(D) OTHER INFORMATION:/note= "May be C or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:22
(D) OTHER INFORMATION:/note= "May be T or C"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:23
(D) OTHER INFORMATION:/note= "May be G or T"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:24
(D) OTHER INFORMATION:/note= "May be G, C or A"

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

ATCTGAGGTA TGTTGAAAAG CTGGA 25

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:6
(D) OTHER INFORMATION:/note= "May be C or A"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:15
(D) OTHER INFORMATION:/note= "May be C or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:21
(D) OTHER INFORMATION:/note= "May be C or T"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:24
(D) OTHER INFORMATION:/note= "May be C or T"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:27
(D) OTHER INFORMATION:/note= "May be C or A"

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

TTCACCATGT ACACCACCTG CATCATCTGG 30

(2) INFORMATION FOR SEQ ID NO: 4:

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

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:9
(D) OTHER INFORMATION:/note= "May be C or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:12
(D) OTHER INFORMATION:/note= "May be A or C"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:15
(D) OTHER INFORMATION:/note= "May be C or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:23
(D) OTHER INFORMATION:/note= "May be C or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:24
(D) OTHER INFORMATION:/note= "May be C or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:27
(D) OTHER INFORMATION:/note= "May be C or T"

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

AGCGATGACG AAGACGACTC CACCACCTC 29

(2) INFORMATION FOR SEQ ID NO: 5:

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

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:6
(D) OTHER INFORMATION:/mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:9
(D) OTHER INFORMATION:/note= "MAY BE A OR G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:11
(D) OTHER INFORMATION:/mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:12
(D) OTHER INFORMATION:/mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:15
(D) OTHER INFORMATION:/mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:16
(D) OTHER INFORMATION:/note= "May be A or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:18
(D) OTHER INFORMATION:/note= "May be G or T"

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

TATGGGGAAA GGGGGATGGA 20

(2) INFORMATION FOR SEQ ID NO: 6:

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

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:2
(D) OTHER INFORMATION:/mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:5
(D) OTHER INFORMATION:/mod_base= i

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:8
(D) OTHER INFORMATION:/note= "May be A or G"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:11
(D) OTHER INFORMATION:/note= "May be A or C"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:14
(D) OTHER INFORMATION:/note= "May be C or T"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:17
(D) OTHER INFORMATION:/mod_base= i

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

TGACGAAAAC AAACCCGAT 19

(2) INFORMATION FOR SEQ ID NO: 7:

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

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:6
(D) OTHER INFORMATION:/note= "May be G or C"

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:15
(D) OTHER INFORMATION:/note= "May be A, G or T"

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

CACGTGGTGT ACATAGTGAA 20