

[57] Abstract

This invention is related to rat cerebellum derived G protein-coupled receptor protein or partial peptide thereof, or salts thereof, nucleic acid coding for said receptor and derivatives thereof, and the like.

The rat cerebellum derived G protein-coupled receptor protein or nucleic acid coding for this and derivatives thereof of this invention can be used for determination of ligand (agonist) with respect to G protein-coupled receptor protein of this invention, preventative and/or therapeutic agent of disease related to dysfunction of G protein-coupled receptor protein of this invention, genetic diagnostic agent, screening of the compound which changes the quantity of expression of the protein of this invention or partial peptide thereof, and the like.

Specification

Novel G protein-coupled receptor protein and DNA thereof

The field of technology

This invention is related to rat cerebellum derived novel G protein-coupled receptor protein or salts thereof, and DNA coding for this.

Background technology

Many physiologically active substances such as hormone and neurotransmitter regulate function of living matter through specific receptor protein presenting in cell membrane. Most among these receptor proteins carry out signal transduction in cell through activation of coupled guanine nucleotide-binding protein (below, in some cases abbreviated as G protein). Moreover, because they have a common structure having 7 transmembrane regions, they are named generally as G protein-coupled receptor proteins or seven times transmembrane type receptor proteins (7TMR).

G protein-coupled receptor protein is present in each functional cell surface of cell or organ of living matter and has a physiologically important role as a target for a molecule which regulates the functions of those cells and organs, for example hormone, neurotransmitter a physiologically

active substance. The receptor transduces the signal through binding with a physiologically active substance, and various kinds of reactions such as activation and depression of cell are caused by this signal.

The elucidation of the relation of the substance regulating a complicated functions in cells and organs of various living matter and their specific receptor, in particular G protein-coupled receptor protein provides an extremely important means for the understanding of the functions in cells and organs of various living matter and the drug development closely related to these.

For example, in various kinds of organs of living matter, control of physiological function is carried out under the control by many hormones, hormone analogues, neurotransmitters or physiologically active substances. In particular physiologically active substance is present in various sites in living matter, and the control of physiological function thereof is carried out through receptor protein corresponding to each. There are still many unknown hormones and neurotransmitters and other physiologically active substances too in living matter, and there are many ones in which so far the structure of their receptor protein are not reported on either. Furthermore even for the already known receptor proteins, there are many ones which are not understood whether subtypes are present.

The elucidation of the relation of the substance regulating a complicated functions in cells and organs of various living matter and their specific receptor proteins is an extremely important means for the drug development. Moreover in order to efficiently screen agonist and antagonist with respect to receptor protein and to develop drugs, it has been required that the function of genes of the receptor proteins expressed in living matter is elucidated and these are expressed with suitable expression system.

Recently a random analysis of cDNA sequence has been actively carried out as a measure for analysing gene appearing in living matter, and fragment sequence of cDNA which was obtained in this way is registered in a database as Expressed Sequence Tag (EST), and it is released. But many EST is only sequencing information, and it is difficult to estimate function thereof.

In the prior art the substance which hinders binding of G protein-coupled receptor and physiologically active substance (namely, ligand) and the substance which binds and causes

signal transduction in the same way as physiologically active substance (namely, ligand) have been utilised as the drug which regulates living matter function as agonist or the antagonist specific for these receptors. Accordingly it is important in this way in physiological expression in living matter, and also to discover a novel G protein-coupled receptor protein which can be the target for the drug development and to clone the gene thereof (for example cDNA) becomes an important means for discovering specific ligand, agonist and antagonist of novel G protein-coupled receptor protein.

But, as for G protein-coupled receptor, all of them have not been discovered, there are many unknown G protein-coupled receptors, and so-called orphan receptors wherein corresponding ligands have not been identified, and a search of new G protein-coupled receptors and elucidation of the function are desired earnestly at present.

G protein-coupled receptor is useful for the search of new physiologically active substance (namely, ligand) using signal transduction action thereof as indicator and a search of antagonist or agonist. On the other hand, even if physiological ligand is not discovered, antagonist or agonist can be produced with respect to said receptor by analysing physiological effect of said receptor from inactivation experiment of said receptor (knockout animal). These ligand, agonist and antagonist with respect to said receptor can be expected to be utilised as prevention / treatment drug and diagnosis drug of disease related to dysfunction of G protein-coupled receptor.

Moreover there is often the case where the lowering or accentuation of function of said receptor in living matter on the basis of gene mutation of G protein-coupled receptor is the cause of some diseases. In this case, in addition to administration of agonist or antagonist with respect to said receptor, gene therapy by introduction of said receptor gene into living matter (or one specific organ) or introduction of anti-sense nucleic acid with respect to said receptor gene can be applied. In this case, the base sequence of said receptor is necessary and indispensable information in order to examine presence of deficiency or mutation on gene, and the gene of said receptor can be applied to prevention / treatment drug and diagnosis drug of disease to participate in dysfunction of said receptor.

Indication of invention

This invention is to put forward a novel G protein-coupled receptor protein which is useful as above. Namely, G protein-coupled receptor protein or partial peptide thereof, or salts thereof, polynucleotide (DNA, RNA and derivatives thereof) containing polynucleotide (DNA, RNA and derivatives thereof) coding for said receptor protein or partial peptide thereof, recombinant vector containing said polynucleotide, a transformant retaining said recombinant vector, a process for the production of said G protein-coupled receptor protein or salts thereof, an antibody with respect to said G protein-coupled receptor protein or partial peptide thereof or salts thereof, a compound or salt thereof which changes the quantity of expression of said G protein-coupled receptor protein, a process for the determination of ligand with respect to said G protein-coupled receptor protein, screening method/ screening kit for a compound (antagonist, agonist) or salts thereof that changes the binding of the ligand and said G protein-coupled receptor protein, a compound or salt thereof that changes the binding of the ligand and said G protein-coupled receptor protein, and medicine containing the compound or salts thereof that changes the binding of the ligand and said G protein-coupled receptor protein or the compound or salts thereof which changes the quantity of expression of said G protein-coupled receptor protein, and the like are put forward.

These inventors, as a result of having repeated assiduous investigations, isolated cDNA which coded for rat cerebellum derived novel G protein-coupled receptor protein based on EST information made by degenerated PCR method, and successfully analysed total base sequence thereof. And this base sequence was translated into amino acid sequence, and the first to the seventh transmembrane regions were confirmed on a hydrophobic plot, and that proteins coded in cDNA of this were confirmed as seven times transmembrane type G protein-coupled receptor proteins. These inventors repeated investigation based on these insight, and as a result this invention was completed.

In other words, this invention is related to,

[1] G protein-coupled receptor protein or salts thereof characterised by containing amino acid sequence of the same or substantially same as the amino acid sequence represented by sequence number: 1,

- [2] Partial peptide or salts thereof of G protein-coupled receptor protein in accordance with aforesaid [1],
- [3] Polynucleotide containing the polynucleotide having base sequence coding for the G protein-coupled receptor protein in accordance with aforesaid [1],
- [4] Polynucleotide in accordance with aforesaid [3] that is DNA,
- [5] Polynucleotide in accordance with aforesaid [3] to have base sequence represented by sequence number: 2,
- [6] Recombinant vector containing polynucleotide in accordance with aforesaid [3],
- [7] Transformant transformed with recombinant vector in accordance with aforesaid [6],
- [8] A process for the production of G protein-coupled receptor protein in accordance with aforesaid [1] to be characterised in that transformant in accordance with aforesaid [7] is cultured and G protein-coupled receptor protein in accordance with aforesaid [1] is formed,
- [9] Antibody with respect to G protein-coupled receptor protein in accordance with aforesaid [1] or partial peptide or salts thereof in accordance with aforesaid [2],
- [10] Antibody in accordance with aforesaid [9] that is a neutralising antibody inactivating signal transduction of G protein-coupled receptor protein in accordance with aforesaid [1],
- [11] Diagnosis drug obtained by containing antibody in accordance with aforesaid [9],
- [12] Ligand with respect to G protein-coupled receptor protein in accordance with aforesaid [1] or the salts thereof which can be obtained by using G protein-coupled receptor protein in accordance with aforesaid [1] or partial peptide or salts thereof in accordance with aforesaid [2],
- [13] Medicine obtained by containing ligand of G protein-coupled receptor protein in accordance with aforesaid [12],
- [14] Determination method of ligand with respect to G protein-coupled receptor protein in accordance with aforesaid [1] to be characterised in that G protein-coupled receptor protein in accordance with aforesaid [1] or partial peptide or salts thereof in accordance with aforesaid [2] are used,
- [15] Screening process of ligand or salts thereof and compound or salts thereof to change binding of G protein-coupled receptor protein in accordance with aforesaid [1] characterised in that G protein-coupled receptor protein in accordance with aforesaid [1] or partial peptide or salts thereof in accordance with aforesaid [2] are used,
- [16] Kit for screening of compound or salts thereof to change the binding of ligand and G protein-coupled receptor protein in accordance with aforesaid [1] characterised by containing G protein-

coupled receptor protein in accordance with aforesaid [1] or partial peptide or salts thereof in accordance with aforesaid [2],

[17] The compound and salts thereof to change the binding of ligand and G protein-coupled receptor protein in accordance with aforesaid [1] that can be obtained using a screening process in accordance with aforesaid [15] or a kit for screening in accordance with aforesaid [16],

[18] Medicine obtained by containing the compound or salts thereof to change ligand and binding of G protein-coupled receptor protein in accordance with aforesaid [1] that can be obtained using a screening process in accordance with aforesaid [15] or a kit for screening in accordance with aforesaid [16],

[19] Polynucleotide that hybridises with polynucleotide in accordance with aforesaid [3] under highly stringent condition,

[20] Polynucleotide that contains base sequence complementary to polynucleotide in accordance with aforesaid [3] or a part thereof,

[21] Determination method of mRNA of G protein-coupled receptor protein in accordance with aforesaid [1] to be characterised in that polynucleotide in accordance with aforesaid [3] or a part thereof is used,

[22] Determination method of G protein-coupled receptor protein in accordance with aforesaid [1] to be characterised in that antibody of aforesaid [9] is used,

[23] Diagnosis method of disease related to the function of G protein-coupled receptor protein in accordance with aforesaid [1] to be characterised in that determination method of aforesaid [21] or [22] is used,

[24] Screening process of compound or salt thereof to change the quantity of expression of G protein-coupled receptor protein in accordance with aforesaid [1] to be characterised in that determination method of aforesaid [21] is used,

[25] Screening process of compound or salt thereof to change the quantity of G protein-coupled receptor protein in accordance with aforesaid [1] on cell membrane to be characterised in that determination method of aforesaid [22] is used,

[26] Compound or salt thereof to change the quantity of expression of G protein-coupled receptor protein in accordance with aforesaid [1] that can be obtained by using the screening process of aforesaid [24],

[27] Compound or salt thereof to change the quantity of G protein-coupled receptor protein in accordance with aforesaid [1] on cell membrane that can be obtained by using the screening process of aforesaid [25].

Furthermore it puts forward,

[28] G protein-coupled receptor protein in accordance with aforesaid [1] or salts thereof wherein the protein containing, (1) the amino acid sequence represented by sequence number: 1 or amino-acid represented by sequence number: 1 in which one or more amino acids (preferably around 1-30, more preferably around 1-9, and more preferably several (1 or 5)) are deleted, (2) the amino acid sequence represented by sequence number: 1 in which one or more amino acids (preferably around 1-30, more preferably around 1-10, and more preferably several (1 or 5)) are added, (3) amino-acid represented by sequence number: 1 in which one or more amino acids (preferably around 1-30, more preferably around 1-10, and more preferably several (1 or 5)) are substituted with other amino acids, or (4) amino acid sequence comprising a combination of these,

[29] Determination method of ligand of aforesaid [14] to be characterised in that G protein-coupled receptor protein in accordance with aforesaid [1] or salt thereof or partial peptide or salt thereof in accordance with aforesaid [2] is made contact with a test compound,

[30] Determination of ligand in accordance with aforesaid [29] wherein the ligands comprise for example angiotensin, bombesin, cannabinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal polypeptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related peptide), leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenalin, and - chemokine (chemokine) [for example IL-8, GRO β , GRO γ , GRO α , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1 α , MIP-1 β , RANTES], endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptide, gallanin,

[31] Screening process in accordance with aforesaid [15] to be characterised in that [i] G protein-coupled receptor protein in accordance with aforesaid [1] or salts thereof or partial peptide or salts thereof in accordance with aforesaid [2] and ligand are contacted and [ii] G protein-coupled receptor protein in accordance with aforesaid [1] or salts thereof or partial peptide or salts thereof in accordance with aforesaid [2], ligand and a test compound are contacted and comparison is made,

[32] Screening process of the compound or salts thereof to change binding of ligand and G protein-coupled receptor protein in accordance with aforesaid [1] to be characterised in that [i] a labelled ligand is contacted with G protein-coupled receptor protein in accordance with aforesaid [1] or salts thereof or partial peptide or salts thereof in accordance with aforesaid [2] and ligand are contacted and [ii] a labelled ligand and test compound are contacted with G protein-coupled receptor protein in accordance with aforesaid [1] or salts thereof or partial peptide or salts thereof in accordance with aforesaid [2], the quantity of binding of labelled ligand with respect to G protein-coupled receptor protein in accordance with aforesaid [1] or partial peptide in accordance with aforesaid [3] or amide thereof or ester thereof or salts thereof is measured and comparison is made,

[33] Screening process of the compound or salts thereof to change binding of ligand and G protein-coupled receptor protein in accordance with aforesaid [1] to be characterised in that [i] a labelled ligand is contacted with a cell containing G protein-coupled receptor protein in accordance with aforesaid [1] are contacted and [ii] a labelled ligand and test compound are contacted with a cell containing G protein-coupled receptor protein in accordance with aforesaid [1], the quantity of binding of labelled ligand with respect to the cell is measured and comparison is made,

[34] Screening process of the compound or salts thereof to change binding of ligand and G protein-coupled receptor protein in accordance with aforesaid [1] to be characterised in that [i] a labelled ligand is contacted with a membrane fraction of the cell containing G protein-coupled receptor protein in accordance with aforesaid [1] or salts thereof are contacted and [ii] a labelled ligand and test compound are contacted with a membrane fraction of the cell containing G protein-coupled receptor protein in accordance with aforesaid [1] or salts thereof, the quantity of binding of labelled ligand with respect to the membrane fraction is measured and comparison is made,

[35] Screening process of the compound or salts thereof to change binding of ligand and G protein-coupled receptor protein in accordance with aforesaid [1] to be characterised in that [i] a labelled ligand is contacted to the G protein-coupled receptor protein which has appeared on the cell membrane of said transformant by culturing transformant in accordance with aforesaid [7] and [ii] a labelled ligand and test compound are contacted to the G protein-coupled receptor protein which has appeared on the cell membrane of said transformant by culturing transformant in accordance with aforesaid [7], the quantity of binding of labelled ligand with respect to G

protein-coupled receptor protein in accordance with aforesaid [1] is measured and comparison is made,

[36] Screening process of the compound or salts thereof to change binding of ligand and G protein-coupled receptor protein in accordance with aforesaid [1] to be characterised in that [i] a compound which activates G protein-coupled receptor protein in accordance with aforesaid [1] is contacted a cell containing G protein-coupled receptor protein in accordance with aforesaid [1] and [ii] a compound which activates G protein-coupled receptor protein in accordance with aforesaid [1] and test compound are contacted with a cell containing G protein-coupled receptor protein in accordance with aforesaid [1], the cell stimulation activity through G protein-coupled receptor protein coupled receptor protein is measured and comparison is made,

[37] Screening process of the compound or salts thereof to change binding of ligand and G protein-coupled receptor protein in accordance with aforesaid [1] characterised in that [i] a compound which activates G protein-coupled receptor protein in accordance with aforesaid [1] is contacted to the G protein-coupled receptor protein which has appeared on the cell membrane of said transformant by culturing transformant in accordance with aforesaid [7] and [ii] a compound which activates G protein-coupled receptor protein in accordance with aforesaid [1] and test compound are contacted to the G protein-coupled receptor protein which has appeared on the cell membrane of said transformant by culturing transformant in accordance with aforesaid [7], the cell stimulation activity through the G protein-coupled receptor protein is measured and comparison is made,

[38] Screening process in accordance with [36] aforesaid or aforesaid [37] that the compound activating G protein-coupled receptor protein in accordance with aforesaid [1] comprises angiotensin, bombesin, cannabinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal polypeptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related peptide), leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenalin, and - chemokine (chemokine) [for example IL-8, GRO₁, GRO₂, GRO₃, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1₁, MIP-1₂, RANTES], endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptide, gallanin

[39] The compound or salts thereof to change binding of ligand and G protein-coupled receptor protein in accordance with aforesaid [1] that can be obtained with screening process in accordance with aforesaid [31]-[38],

[40] Medicine characterised by containing the compound or the salts thereof to change binding of ligand and G protein-coupled receptor protein in accordance with aforesaid [1] which can be obtained with screening process in accordance with aforesaid [31]-[38],

[41] Kit for screening in accordance with aforesaid [16] to contain cell containing G protein-coupled receptor protein in accordance with aforesaid [1],

[42] Kit for screening in accordance with aforesaid [16] to be characterised by containing the membrane fraction of cell containing G protein-coupled receptor protein in accordance with aforesaid [1],

[43] Kit for screening in accordance with aforesaid [16] to be characterised by containing the G protein-coupled receptor protein expressed in cell membrane of said transformant by being cultured transformant in accordance with aforesaid [7],

[44] The compound or salts thereof to change binding of ligand and G protein-coupled receptor protein in accordance with aforesaid [1] that can be obtained using a kit for screening in accordance with aforesaid [41]-[43],

[45] Medicine characterised by containing the compound or salts thereof to change binding of ligand and G protein-coupled receptor protein in accordance with aforesaid [1] that can be obtained using a kit for screening in accordance with aforesaid [41]-[43],

[46] Assay of G protein-coupled receptor protein in accordance with aforesaid [1], partial peptide or salts thereof in accordance with [2] characterised in antibody in accordance with aforesaid [9] and G protein-coupled receptor protein in accordance with aforesaid [1] or partial peptide or salts thereof in accordance with aforesaid [2] are contacted,

[47] Assay of G protein-coupled receptor protein in accordance with aforesaid [1], partial peptide or salts thereof in accordance with [2] in a test solution characterised in that antibody in accordance with aforesaid [9], test solution and labelled G protein-coupled receptor protein in accordance with aforesaid [1] or partial peptide or salts thereof in accordance with aforesaid [2] are competitively reacted, and the ratio of labelled G protein-coupled receptor protein in accordance with aforesaid [1] or partial peptide or salts thereof in accordance with aforesaid [2] which is bound to said antibody is measured, and

[48] Assay of G protein-coupled receptor protein in accordance with aforesaid [1], partial peptide or salts thereof in accordance with [2] in a test solution characterised in that test solution, antibody in accordance with aforesaid [9] which is insolubilised on a carrier and labelled antibody in accordance with aforesaid [9] are reacted at the same time or successively, thereafter the activity of the labelled agent on the insolubility carrier is measured.

Brief Description of the Figures

Figure 1 shows base sequence of DNA coding for rat cerebellum derived novel G protein-coupled receptor protein rCB7T084 of this invention obtained in Example 1, and amino acid sequence deduced from this (continued to Figure 2).

Figure 2 shows base sequence of DNA coding for rat cerebellum derived novel G protein-coupled receptor protein rCB7T084 of this invention obtained in Example 1, and amino acid sequence deduced from this (following from Figure 1).

Figure 3 shows hydrophobic plot of rat cerebellum derived novel G protein-coupled receptor protein rCB7T084 of this invention which is produced by being based on amino acid sequence shown in Figure 1-Figure 2.

The best form to carry out the invention

G protein-coupled receptor protein of this invention (hereafter abbreviated as receptor protein) is a receptor protein containing the same amino acid sequence represented by sequence number: 1 (amino acid sequence in Figure 1 and Figure 2) or substantially same amino acid sequence.

Receptor protein of this invention, for example, can be a protein derived from every cell (for example splenic cell, nerve cell, glia cell, pancreas cell, bone marrow cell, mesangial cell, Langerhans cell, epidermis cell, epithelial cell, endothelial cell, fibroblast, fibrous cell, muscle cell, adipocyte, immunocyte (example, macrophage, T cell, B cell, natural killer cell, mast cell, neutrophil leucocyte, basophil, acidophilic leucocyte, monocyte), megakaryocyte, synovial cell, chondrocyte, osteocyte, osteoblast, osteoclast, mammary gland cell, hepatic cell or stroma cell or precursor cells or committed stem cell of these or cancer cell) or blood cells of human and mammalian organisms (for example guinea pig, rat, a mouse, a rabbit, a pig, a sheep, bovine, a monkey) or from every tissue in which these cells exist, for example brain, each part of brain (nucleus, cerebral cortex, medulla oblongata, cerebella, the occiput leaf, the frontal lobe, a temporal lobe, putamen, caudate, rostrum of callous corpus, nigra), medulla spinalis, hypophysis, ventriculus, pancreas, kidney, a liver, gonad, thyroid gland, cholecyst, bone marrow, adrenal,

skin, a muscle, lung, alimentary canal (example, the large intestine, small intestine), blood vessel, heart, thymus, spleen, a submaxillary gland, tip blood, tip blood corpuscle, glandula prostatica, the testicles, a spermary, ovaria, placenta, the womb, bone, joint, skeletal muscle (in particular, brain and each part of brain) and may be moreover a synthetic protein.

As amino acid sequence having substantially same as amino acid sequence represented by sequence number: 1, for example, amino acid sequence having homology with amino acid sequence represented by sequence number: 1 by about 50 % or more, preferably about 70 % or more, more preferably about 80 % or more, more preferably about 90 % or more, and most preferably about 95 % or more homology is proposed.

As protein containing the amino acid sequence having substantially same as amino acid sequence represented by sequence number: 1, for example, a protein having substantially same as amino acid sequence represented by sequence number: 1 and also having activity of substantially same quality as amino acid sequence represented by sequence number: 1 is preferred.

As the activity of substantially same quality, for example, ligand avidity, signal transduction action are nominated. The substantially same quality shows that those activities are of the same nature. Accordingly it is preferred that ligand avidity and activity such as signal transduction action are equality (example, about 0.01-100 times, preferably about 0.5-20 times, more preferably about 0.5-2 times), but quantitative element such as molecular weight of protein and degree of activity of these may be different.

Measurement of ligand binding avidity and activity such as signal transduction action can be carried out in accordance with itself familiar process, but for example it can be measured according to determination method and screening process of ligand mentioned later.

Moreover, as receptor protein of this invention, proteins containing (1) amino acid sequence represented by sequence number: 1 in which one or more amino acids (preferably around 1-30, more preferably around 1-9, and more preferably several (1 or 5)) are deleted, (2) amino acid sequence represented by sequence number: 1 in which one or more amino acids (preferably around 1-30, more preferably around 1-10, and more preferably several (1 or 5)) are added, (3)

amino acid sequence represented by sequence number: 1 in which one or more amino acids (preferably around 1-30, more preferably around 1-10, and more preferably several (1 or 5)) are substituted with other amino acids, or (4) amino acid sequence comprising a combination of these are also used.

As for the receptor protein in this specification, according to convention of peptide representation, the left-side end is N terminal (amino terminus) and the right-side end is C terminal (carboxyl terminus). The receptor protein of this invention including receptor protein containing the amino acid sequence which is represented by sequence number: 1 usually has carboxyl group (-COOH) or carboxylate (-COO-) at C terminal, however, the C terminal may be amide (-CONH₂) or ester (-COOR).

Wherein, as R in ester, for example C1-6 alkyl group such as methyl, ethyl, n-propyl, isopropyl or n-butyl, C3-8 cycloalkyl group for example cyclo pentyl, cyclohexyl, C6-12 aryl group such as phenyl, -naphthyl, phenyl-C1-2 alkyl group for example benzyl, phenethyl, C7-14 aralkyl group such as -naphthyl-C1-2 alkyl group such as -naphthylmethyl, in addition, pivaloyl oxymethyl group used widely as orally administered ester thereof are used.

When receptor protein of this invention has carboxyl group (or carboxylate) besides C terminal, the one in which carboxyl group is ester or amide thereof is contained in receptor protein of this invention, too. In this case, as ester thereof, for example, ester of aforesaid C terminal is used.

Furthermore receptor protein of this invention includes one in which amino group of methionine residue of N terminal in aforesaid protein is protected with protecting group (for example C1-6 acyl group such as C2-6 alkanoyl group such as formyl group, acetyl), one in which N-terminal side was cut off in-vivo and formed glutamyl group is formed into pyroglutamic acid, one in which the substituent on side-chain of amino-acid in a molecule (for example -OH, -SH, COOH, amino group, imidazole group, indole group, guanidino group) are protected with a suitable protective groups (for example C1-6 acyl group such as C2-6 alkanoyl group such as formyl group, acetyl), or one in which conjugate protein such as so-called glycoprotein that sugar chain is bonded.

For example, as embodiment of receptor protein of this invention, receptor proteins derived from rat (more preferably derived from rat cerebellum) containing the amino acid sequence which is represented by sequence number: 1 are used.

As partial peptide of receptor protein of this invention (below in some cases abbreviated as partial peptide), any partial peptide of aforesaid receptor protein of this invention can be used, and for example, among receptor protein molecules of this invention, one which is a site exposed to the outside cell membrane, and having receptor activity is used.

As embodiments as partial peptide of the receptor protein having the amino acid sequence represented by sequence number: 1 it is peptide including the section where it was analysed in the hydrophobic plot analysis represented by Figure 3 as extracellular region (hydrophilic site). Moreover peptide containing hydrophobic site in part is possible to use in the same way, too. The peptide which separately include individual domains can be use, too, but even peptide of the section that simultaneously includes plurality of domains can be used.

As for the number of amino-acid of partial peptide of this invention, peptide having amino acid sequence of at least 20 or more, preferably 50 or more, or more preferably 100 or more is preferred among constituent amino acid sequence of receptor protein of aforesaid this invention.

Amino acid sequence of the substantially same shows this amino acid sequence and about 50 % or more preferably about 70 % or more, more preferably about 80 % or more amino acid sequence which more preferably have about 90 % or more, and most preferably about 95 % or more homology.

Wherein "the activity of substantially same quality" denotes the same meaning as mentioned above. Measurement of "activity of substantially same quality" can be carried out in the same way as in an item mentioned above.

Moreover the partial peptide of this invention may be deficient in 1 or more amino-acids (preferably 1-10, or more preferably several (1-5)) in aforesaid amino acid sequence or added with 1 or more amino-acids (preferably 1-20, more preferably 1-10 or even more preferably

several (1-5)) in amino acid sequence thereof, or substituted 1 or more amino-acids (preferably 1-10, or more preferably several (1-5)) in amino acid sequence thereof with other amino acids.

Moreover C-terminal is usually carboxyl group (-COOH) or carboxylate (-COO-) for the partial peptide of this invention, but C terminal can be amide (-CONH₂) or ester (-COOR) as in the aforesaid receptor protein of this invention.

Furthermore the one which amino group of methionine residue of N terminal is protected with a protecting group, in the same way as in receptor protein of aforesaid this invention the one where N-terminal side was cut off in in-vivo, and formed Gln is formed into pyroglutamic acid, one protected with the protecting group that the substituent on side-chain of amino-acid in a molecule or conjugate peptides such as so-called glycopeptide in which sugar chain is bonded are included in the partial peptide of this invention.

Moreover C-terminal is usually carboxyl group (-COOH) or carboxylate (-COO-) for the partial peptide of this invention, but C terminal can be amide (-CONH₂) or ester (-COOR) as in the aforesaid receptor protein of this invention.

As salt of receptor-protein of this invention or partial peptide thereof, acid or physiologically acceptable salt with the base is used, and the more particularly pharmacologically acceptable acid addition salt is preferred. As such salt, a salt for example with inorganic acid (for example hydrochloric acid, phosphoric acid, hydrobromic acid, sulphuric acid) or a salt with organic acid (for example acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulphonic acid, benzene sulphonic acid) is used.

Receptor protein of this invention or salts thereof can be produced by itself well-known process for purification of receptor protein from tissue or cell of human and mammalian organisms mentioned above and can be produced by culturing transformant containing DNA coding for receptor protein of this invention mentioned later. Moreover it can be produced by the protein synthesis method mentioned later or a method in accordance with this.

When produced from tissue or cell of human and mammalian organisms, the tissue or cell of human and mammalian organisms is homogenised next it is extracted with acid and purification and isolation can be carried out from the said extract by combining reversed-phase chromatography, chromatography such as ion exchange chromatography.

In synthesis of receptor protein of this invention, partial peptide thereof or salts thereof, it is possible to use usual commercial resin for protein synthesis. For example, as resin such as chloromethyl resin, hydroxymethyl resin, benzhydrylamine resin, aminomethyl resin, 4-benzyl oxy benzyl alcohol resin, 4-methyl benzhydrylamine resin, PAM resin, 4-hydroxymethyl methylphenyl acetamide thereof methyl resin, polyacrylamide thereof resin, 4-(2',4'-dimethoxyphenyl-hydroxy methyl) phenoxy resin, 4-(2',4'-dimethoxyphenyl-Fmoc amino ethyl) phenoxy resin can be nominated. Using such resin, the amino-acid which protected suitably α -amino group and side-chain functional group is condensed on resin according to various condensation method of itself well-known in order of the sequence of the target protein. Various protecting groups are eliminated at the same time of excising protein from resin in the last of reaction, and furthermore intramolecular disulphide binding reaction is carried out with high dilution solution, and target protein or their amide body is acquired.

On condensation of aforesaid protected amino acid, various activated agent usable for protein synthesis can be used, but carbodiimide species are in particular good. As carbodiimides, DCC, N,N-diisopropyl carbodiimide, N-ethyl-N'-(3-dimethylamino prolyl) carbodiimide are used. For activation by these, protected amino acid is directly added to resin together with additive racemisation inhibiting agent (for example, HOBt, HOObt) or activation of protected amino acid is carried out beforehand as symmetry acid anhydride or HOBt ester thereof or HOObt ester thereof, thereafter, it can be added to the resin.

As the solvent used in activation of protected amino acid and condensation of resin, it is suitably possibly selected from the solvent which is known to be used in protein condensation reaction. For example, acid amide species such as N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidone, halogenated hydrocarbon species such as methylene chloride, chloroform, an alcohol such as trifluoroethanol, sulphoxides such as dimethylsulphoxide, pyridine, ether such as dioxane, tetrahydrofuran, nitriles such as acetonitrile, propionitrile, ester thereof such as methyl acetate, ethyl acetate or suitable mixture of this is used. The reaction temperature is suitably

selected from the range that is known to be used for protein binding forming reactions, and it is suitably selected usually from about -20 degrees to 50 degrees range. Activated amino-acid derivative is used usually by 1.5-4 times excess. As a result of test using ninhydrin reaction, when the condensation is not enough, sufficient condensation can be carried out by repeating condensation reaction without elimination of protecting group. When sufficient condensation is not obtained even if reaction is repeated, unreacted amino-acid can be acetylated using acetylimidazole or an acetic anhydride.

As protecting group of amino group of a starting material, for example, Z, Boc, tertiary pentyloxy carbonyl, isobornyl oxycarbonyl, 4-methoxybenzyl oxycarbonyl, Cl-Z, Br-Z, adamantyl oxycarbonyl, trifluoroacetyl, phthaloyl, formyl, 2-nitrophenylsulphenyl, di phenyl phosphinothioyl, Fmoc are used.

For example, carboxyl group can be protected by alkyl esterification (for example straight chain, branched or cyclic alkyl esterification such as methyl, ethyl, propyl, butyl, t-butyl, cyclo pentyl, cyclohexyl, cyclo heptyl, cyclo octyl, 2-adamantyl), aralkyl esterification (for example benzyl ester, 4-nitrobenzyl ester, 4-methoxybenzyl ester, 4-chlorobenzyl ester, benzhydryl esterification), phenacyl esterification, benzyloxycarbonyl hydrazide formation, t-butoxy carbonyl hydrazide formation, trityl hydrazide formation.

For example, hydroxy group of serine can be protected by etherification or esterification. For example, as group suited for this esterification, the group that derived from lower alkanoyl group such as acetyl group, aroyl group such as benzoyl group, and group derived from carbonic acid such as benzyloxy carbonyl group, ethoxycarbonyl group are used. Moreover for example, as group suited for etherification, it is benzyl group, tetrahydropyranyl group, t-butyl group.

For example, as protecting group of phenolic hydroxy group of tyrosine, BZl, Cl₂-Bzl, 2-nitrobenzyl, Br-Z, tertiary butyl are used.

For example, as protecting group of imidazole of histidine, Tos, 4-methoxy-2,3,6-trimethyl benzenesulphonyl, DNP, benzyloxymethyl, Bum, Boc, Trt, Fmoc are used.

As activated form of carboxyl group of a starting material, for example, corresponding acid anhydride and azide, active ester [ester of alcohol (for example pentachlorophenol, 2,4,5-trichlorophenol, 2,4-dinitrophenol, cyanomethyl alcohol, para nitrophenol, HONB, N-hydroxysuccinimide, N-hydroxyphthalimide, HOBt)] are used. As activated form of amino group of a starting material, for example, corresponding phosphoric acid amide is used.

As elimination of protecting groups (elimination) method, catalytic reduction in the hydrogen stream in the presence of catalyst such as for example Pd-black or Pd-carbon, moreover acid treatment by anhydrous hydrogen fluoride methanesulphonic acid, trifluoromethane sulphonic acid, trifluoroacetic acid or these liquid mixture, base treatment by diisopropyl ethylamine, triethylamine, piperidine, piperazine, or moreover reduction by sodium in liquid ammonia, too are employed. Generally elimination reaction by aforesaid acid treatment is carried out at about -20 degrees to 40 degrees temperatures, but, in acid treatment, addition of cation scavenger such as for example anisole, phenol, thioanisole, meth creosol, rose creosol, dimethylsulphide, 1,4-butane dithiol, 1,2-ethanedithiol is effective. Moreover 2,4-dinitrophenyl group which is used as imidazole protecting group of histidine is eliminated by treatment with thiophenol, and formyl group used as indole protecting group of triptophan is eliminated by alkali treatment due to dilute sodium hydroxide solution, dilute ammonia besides deprotection by acid treatment in the presence of such as aforesaid 1,2-ethanedithiol, 1,4-butane dithiol.

The protection of the functional group which should not participate in reaction of a starting material and protecting group thereof, elimination of protecting group and activation of functional group participating in reaction can be suitably selected from well known groups or well known means.

As another method for obtaining amide of protein, for example, first α -carboxyl group of carboxy terminal amino-acid is amidated and protected, and next the desired chain length for peptide (protein) chain in the amino group side is extended, thereafter, protein in which only the protecting group of carboxyl group at C terminal is eliminated and protein in which only protecting group of α -amino group of N terminal of said peptide chain is eliminated, are produced, and both proteins are condensed in a mixed solvent such as aforesaid one. The details of condensation reaction is similar to the above. All protecting groups are eliminated by the aforesaid method after having been purified by the protection protein which was obtained by condensation and are

possible to obtain desired crude protein. This crude protein is refined using already known various purification technique freely and is possible to obtain amide body of desired protein by freeze-drying the major fraction.

In order to obtain the ester form of protein, for example, -carboxyl group of carboxy terminal amino-acid is condensed with a desired alcohol, and amino acid ester thereof is made and it is possible to obtain desired ester thereof of the protein next in the same way as in amide body of protein.

The partial peptide of protein of this invention or salts thereof can be produced by according to method for the synthesis of peptide of itself well-known or cleaving the protein of this invention with a suitable peptidase. For example, as method for the synthesis of peptide, it may use either a solid-phase synthesis method, or a liquid phase method. In other words partial peptide or amino-acid and the remaining part which can constitute receptor protein of this invention are condensed, and target peptide can be produced by eliminating the protecting group when the product has a protecting group. As well known condensation method and elimination of protecting group, for example, methods in accordance with following (1)-(5) are nominated.

(1) M Bodanszky and M.A. Ondetti, peptide synthesis (Peptide Synthesis), Interscience Publishers, New York (1966)

(2) Schroeder and Lueoke, The peptide (The Peptide), Academic Press, New York (1965)

(3) Shinobu Izumiya et al. Foundation and test of peptide synthesis, Maruzen Co. (1975)

(4) Haruaki Yajima and Shunpei Sakakibara, Bbiochemistry Experiment Course 1, Chemistry of proteins IV, 205 (in 1977)

(5) Haruaki Yajima Ed., Development of Drugs, sequel, Vol. 14, Peptide synthesis, Hirokawa Shoten.

On completion of the reaction, partial peptide of this invention can be purified and isolated by combination of ordinary purification methods, for example, solvent extraction / distillation / column chromatography / liquid chromatography / recrystallisation. When the partial peptide obtained with the aforesaid method is an educt, it can be converted into suitable salt by well known method, conversely when it is obtained as salt it can be converted into educt by well known method.

As for polynucleotide coding for receptor protein of this invention, it can be any kind so long as it contains base sequence coding for aforesaid receptor protein of this invention (DNA or RNA, preferably DNA). As said polynucleotide, it is DNA coding for receptor protein of this invention, RNA such as mRNA, and can be double stranded or single stranded. When it is double stranded, even double stranded DNA, double stranded RNA or hybrid of DNA:RNA is good. When it is single stranded, it can be sense chain (namely, coding chain) or anti-sense chain (namely, non-coding chain).

For example, using polynucleotide coding receptor protein of this invention, it is possible to determine mRNA of receptor protein of this invention by the well known method described in Experimental Medicine Supplement "New PCR and its Application" 15 [7], 1997 or method in accordance with this.

As DNA coding for receptor protein of this invention, it can be any of genomic DNA, genomic DNA library, aforesaid cDNA derived from cell / tissue, cDNA library derived from aforesaid cell / tissue, synthetic DNA. Vector used in a library may be any of bacteriophage, plasmid, cosmid, phagemid. Moreover using the one that was prepared by mRNA fraction or total RNA from aforesaid cell / tissue, it can be directly amplified by Reverse Transcriptase Polymerase Chain Reaction (hereafter, abbreviated as RT-PCR method).

As embodiments as DNA coding for receptor protein of this invention, it can be any, for example, DNA containing the base sequence represented by sequence number: 2 or DNA having base sequence which hybridises with base sequence represented by sequence number: 2 under highly stringent conditions and codes for a protein having substantially the same quality of activity (example, ligand avidity, signal transduction action) as receptor protein of this invention.

As for DNA which can be hybridised with the base sequence represented by sequence number: 2, for example, DNA containing the base sequence which is homologous to the base sequence represented by sequence number: 2 by about 70 % or more, preferably about 80 % or more, more preferably about 90 % or more, and most preferably about 95 % or more is used.

Hybridisation can be carried out according to itself familiar process or a method based on this, for example, a method described in Molecular Cloning, 2nd (J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989). Moreover it can be carried out according to the methods in the attached instructions when a commercial library is used. More preferably it can be carried out under a highly stringent condition.

Said highly stringent condition, for example, represents conditions of about 19-40 mM sodium concentration or preferably 19-20 mM, and, temperature of about 50-70 degrees, preferably about 60-65 degrees. In particular in the case of about 19 mM sodium concentration and temperature of about 65 degrees is most preferred.

More as embodiments, as DNA coding for receptor protein containing the amino acid sequence represented by sequence number: 1, DNA having the base sequence represented by sequence number: 2 is used.

Polynucleotide containing a part of base sequence of DNA coding for receptor protein of this invention or a part of base sequence complementary to said DNA does not only include the DNA coding for partial peptide of this invention described below, but also is used in meaning to include RNA.

In accordance with this invention, anti-sense polynucleotide (nucleic acid) which can inhibit the replication or expression of the receptor G protein-coupled receptor protein gene of this invention can be designed based on the base sequence information of DNA coding for the cloned or determined G protein-coupled receptor protein, and can be synthesised. Such polynucleotide (nucleic acid) can hybridise with RNA of the G protein-coupled receptor protein gene, and can inhibit the synthesis or the function of said RNA, or can regulate and control the expression of G protein-coupled receptor protein gene through interaction of G protein-coupled receptor protein-related RNA. The polynucleotide which is complementary to the sequence selected for G protein-coupled receptor protein related RNA and the polynucleotide which can hybridise specifically with G protein-coupled receptor protein-related RNA, is useful for regulating or controlling the expression of G protein-coupled receptor protein gene in-vivo and in-vitro and is useful for the diagnosis, prevention and/or treatment of diseases related to dysfunction or deficiency of said G protein-coupled receptor protein. A term "corresponding"

means that it is homologous or complementary to a specific sequence of nucleic acid, base sequence or nucleotide including gene. And "corresponding" between nucleotide, nucleic acid or base sequence and peptide (protein) normally indicates amino-acid of a commanded peptide (protein) that it derived from sequence of nucleotide (nucleic acid) or complementary body thereof. G protein-coupled receptor protein gene 5' end hair pin loop, 5' end 6-base pair repeat, 5' end non-translation region, polypeptide translation initiation codon, protein coding region, ORF translation initiation codon, 3' end non-translation region, 3' end palindrome region and 3' end hairpin loop can be selected as preferred target region, but any region in G protein-coupled receptor protein gene can be selected as the target.

Relation of the target nucleic acid and the polynucleotide which is complementary at least to a part of the target region can be said that the relation of the polynucleotide that can hybridise with target material is "anti-sense". As anti-sense polynucleotide, polydeoxynucleotide containing 2-deoxy-D-ribose, polydeoxynucleotide containing D-ribose other type of polynucleotides comprising N-glycoside of purine or pyrimidine base, or other polymers having non-nucleotide backbone (for example, a commercial protein nucleic acid and a nucleic acid polymer specific to synthetic sequence) or other polymers containing special bindings (wherein said polymer contains nucleotide having sequence permitting the pairing of the base or adhesion of base which can be found in DNA and RNA) are proposed. These can be double stranded DNA, single stranded DNA, double stranded RNA, single stranded RNA and furthermore DNA:RNA hybrid, furthermore they can be unmodified polynucleotide (or non-modification oligonucleotide), moreover the one with well known modification, for example one with labels known in the said field, one added with a cap, methylated form, one in which one or more natural nucleotides are substituted with analogues, one with nucleotide modification in a molecule, one having for example uncharged bond (for example methyl phosphonate, phosphotriester, phosphoramidate, carbamate), or having charged bond or having sulphur containing bond (for example phosphorothioate, phosphorodithioate), for example protein (nuclease, nuclease inhibitor, toxin, antibody, signal peptide, poly-L-lysine) and one having side chain group sugar (for example mono saccharide), one having the intercurrent compound (for example acridine, psoralen), one containing chelate compound (for example, metal, boron, oxidising metal, radioactive metal), one containing alkylating agent, or it may be one having modified bond (for example nucleic acid of anomeric form). Wherein "nucleoside", "nucleotide" and "nucleic acid" not only contain purine and pyrimidine base, but also can include one modified with other heterocyclic type base. Such

modification form may include the purine and pyrimidine that are methylated, acylated purine and pyrimidine, or other heterocycle. Also, as for the modified nucleoside and the modified nucleotide, sugar part may be modified, and for example one or more hydroxy groups are substituted at halogen or aliphatic group, or it may be converted into functional group such as ether, amine.

Anti-sense polynucleotide [nucleic acid] of this invention is RNA, DNA or modified nucleic acid (RNA, DNA). As embodiment of modified nucleic acid, sulphur derivative and thiophosphate derivative of nucleic acid and one with resistance to decomposition of poly nucleoside amide thereof and oligo nucleoside amide thereof, are proposed but are not restricted to this. The anti-sense nucleic acid of this invention can be preferably designed by a following policy. In other words anti-sense nucleic acid in cell is made more stable, and cell permeability of anti-sense nucleic acid is raised more, and target makes affinity with respect to the sense chain which is made larger, and toxicity of anti-sense chain is made smaller if it is toxic.

In this way many modifications are known in aforesaid field. It is disclosed in for example J. Kawakami et al., Pharm Tech Japan, Vol. 8, pp. 247, 1992; Vol. 8, pp. 395, 1992; S. T. Crooke et al. ed., Antisense Research and Applications, CRC Press, 1993, and the like.

Anti-sense nucleic acid of this invention can be altered, and modified sugar, base, bond may be contained, it can be given in a special form such as liposome, microsphere, and it is applied by gene treatment, and it can be given in an added form. As one that is used in addition form in this way, polycation body such as the poly lysine which acts to neutralise the charge of phosphoric acid group backbone, or hydrophobic ones such as lipid which increases synergistic action of cell membrane or enhances the incorporation of nucleic acid (for example phospholipid, cholesterol thereof) are proposed. As preferred lipid of addition, cholesterol thereof and derivatives thereof (for example cholesteryl chloro formate, cholic acid) are proposed. Such one can be adhered to 3' end or 5' end of nucleic acid, and it can be adhered through base, sugar, nucleoside bond in a molecule. As other group, one to prevent decomposition due to exonuclease, nuclease such as RNase at group for the cap which is specifically arranged in 3' end of nucleic acid or 5' end are proposed. As group for such a cap, protecting group of hydroxy group made including polyethylene glycol, glycol such as tetraethylene glycol known in the said field are nominated, but are not limited to these.

The inhibition activity of anti-sense nucleic acid can be examined using transformant of this invention, gene expression system of in-vivo and in-vitro of this invention or translation system of in-vivo and in-vitro of receptor protein of this invention. Said nucleic acid can be applied to cell in all kinds of itself well-known methods.

As DNA coding for a partial peptide of this invention, it may be any kind of one as long as it is one containing base sequence coding partial peptide of this invention mentioned above. Moreover but any of genomic DNA, genomic DNA library, cDNA derived from aforesaid cell / tissue, cDNA library derived from aforesaid cell / tissue, synthetic DNA is good. Vector used in a library may come either bacteriophage, plasmid, cosmid, phagemid. Moreover it can be directly amplified by Reverse Transcriptase Polymerase Chain Reaction (hereafter abbreviated as RT-PCR) method using the one prepared by mRNA fraction from aforesaid cell / tissue.

As embodiments as DNA coding for partial peptide of this invention, for example [1] DNA having partial base sequence of DNA having the base sequence represented by sequence number: 2, or [2] DNA having partial base sequence of DNA coding for the receptor proteins which base sequence hybridise with the base sequence represented by sequence number: 2 under highly stringent conditions, and having receptor protein of this invention and the activity of substantially same quality (example, ligand avidity, signal transduction action) is used.

As DNA that can hybridise with the base sequence represented by sequence number: 2, for example, base sequence represented by sequence number: 2 and DNA containing base sequences of about 70 % or more, preferably about 80 % or more, more preferably about 90 % or more, and most preferably about 95 % or more homology are used.

As means of cloning of DNA which completely codes for the receptor protein of this invention or partial peptides thereof (below there are case to be abbreviated receptor protein of this invention), it is amplified by PCR method using synthetic DNA primer having partial base sequence of receptor protein of this invention, and or DNA built in a suitable vector can be selected by hybridisation with using DNA fragment or synthetic DNA coding for total region or some of receptor protein of this invention. Method of hybridisation can be carried out in accordance with, for example Molecular Cloning, 2nd (J. Sambrook et al., Cold Spring Harbor

Lab. Press, 1989). Moreover when a commercial library is used, it can be carried out according to method in accordance with using attached instructions.

Conversion of base sequence of DNA can be carried out according to itself familiar process such as Gapped duplex method and Kunkel method or method according to these, using well known kit, for example Mutan [TM]-G (Takara Shuzo Co.), Mutan [TM]-K (Takara Shuzo Co.).

DNA coding for cloned receptor protein of this invention can be used without further treating or if desired after digested with restriction enzyme or linker is added. Said DNA has ATG as translation initiation codon in 5' terminal side thereof, moreover may have TAG or TAA, TGA as translation termination codon in 3' terminal side. These translation initiation codons and translation termination codon can be added using a suitable synthetic DNA adapter.

The expression vector of the receptor protein of this invention can be produced by for example [a] Target DNA fragment is excised from DNA coding for receptor protein of this invention, [b] Said DNA fragment is connected with the downstream of promoter in suitable expression vector.

As vector, plasmid derived from E. coli (example, pBR322, pBR325, pUC12, pUC13), plasmid derived from Bacillus subtilis (example, pUB110, pTF5, PC194), yeast derived plasmid (example, pSH19, pSH15), bacteriophage such as phage, an animal virus such as retrovirus, vaccinia virus, baculovirus, and others pA1-11, pXT1, pRc/CMV, pRc/RSV, pcDNA1/Neo are used.

As promoter used in this invention, any kind of one is good so long as it is an appropriate promoter corresponding to the host used in genetic expression. For example, SR promoter, SV40 promoter, LTR promoter, CMV promoter, HSV-TK promoter are proposed when animal cell is used as host.

Among these it is preferred to use CMV promoter, SR promoter. When the host is Escherichia genus bacteria, Trp promoter, lac promoter, recA promoter, PL promoter, lpp promoter are preferred, when the host is Bacillus genus bacteria, SPO1 promoter, SPO2 promoter, penP promoter are preferred, when the host is yeast, PHO5 promoter, PGK promoter, GAP bromo-

evening one, ADH promoter are preferred. When the host is insect cell, polyhedrin promoter, P10 promoter are preferred.

In expression vector, it is possible to use in addition to above, one containing as desired enhancer, splicing signal, poly A addition signal, selected marker, SV40 duplicating origin (below there are case to be abbreviated as SV40ori). For example, as selected marker, dihydrofolate reductase (below there are case to be abbreviated as dhfr) gene [methotrexate (MTX) resistance], ampicillin resistance gene (below there are case to be abbreviated as Ampr), neomycin resistance gene (below there are case to be abbreviated as NEO_r, G418 resistance) are nominated. In particular the target gene can be selected by the culture medium which does not include thymidine when dhfr gene is used as selected marker using CHO (dhfr-) cell.

Moreover the signal sequence which matched host is added in accordance with requirements to N-terminal side of receptor protein of this invention. When the host is Escherichia genus bacteria, PhoA / signal sequence, OmpA / signal sequence can be respectively used, when the host is Bacillus genus bacteria, -amylase signal sequence, subtilisin signal sequence can be respectively used, when the host is yeast, MF / signal sequence, SUC2 / signal sequence can be respectively used, when the host is animal cell, insulin signal sequence, -interferon signal sequence, antibody molecule / signal sequence can be respectively used.

Using vector containing DNA coding for receptor protein of this invention that was constructed in this way, transformant can be produced.

As host, for example, Escherichia genus bacteria, Bacillus genus bacteria, yeast, insect cell, insect, animal cell are used.

As embodiment of Escherichia genus bacteria, Escherichia coli K12 / DH1 [Proc. Natl. Acad. Sci. USA, 60 volume ,160 (1968)], JM103 [Nucleic Acids Research, 9 volume, 309 (1981)], JA221 [Journal of Molecular Biology, 120 ,517 (1978)], HB101 [Journal of molecular biology, 41 ,459 (1969)], C600 [Genetics, 39 volume ,440 (1954)] are used.

As Bacillus genus bacteria, for example Bacillus subtilis MIII4 [gene, 24, 255 (1983)], 207-21 [Journal of Biochemistry, 95 volume, 87 (1984)] are used.

As yeast, for example, *Saccharomyces cerevisiae* AH22, AH22R-, NA87-11A, DKD-5D, 20B-12, *Schizosaccharomyces pombe* NCYCI913, NCYC2036, *Pichia pastoris* are used.

As insect cell, for example, when a virus is AcNPV, larva derived established cell (*Spodoptera frugiperda* cell; Sf cell) of *Mamestra brassicae*, MG1 cell derived from a midgut of *Trichoplusia ni*, High Five [TM] cell derived from ovum of *Trichoplusia ni*, cell derived from *Mamestra brassicae* or cell derived from *Estigmene acrea* are used. When a virus is BmNPV, *Bombyx mori* derived established cell (*Bombyx mori* N; BmN cell) or the like is used. As said Sf cell, for example Sf 9 cell (ATCC CRL1711), Sf 21 cell (Vaughn, J, L et al. *In Vivo*, 13, 213-217, (1977)) are used.

As an insect, for example, larvae of a silkworm are used [Maeda et al., *Nature*, 315 volume, 592 (1985)].

As animal cell, for example, monkey cell COS-7, Vero, Chinese hamster cell CHO (hereafter abbreviated as CHO cell), dhfr gene defect Chinese hamster cell CHO (hereafter abbreviated as CHO (dhfr-) cell), mouse L cell, mouse AtT-20, mouse myeloma cell, rat GH3, human FL cell are used.

For example, transformation of *Escherichia* genus bacteria can be carried out according to methods described in *Proc. Natl. Acad. Sci. USA*, 69 volume, 2110 (1972) or *Gene*, 17 volume, 107 (1982). For example, transformation of *Bacillus* genus bacteria can be carried out according to method described in *Molecular & General Genetics*, 168, 111s (1979).

For example, transformation of yeast can be carried out according to methods described in *Methods in Enzymology*, 194 volume, 182-187 (1991), *Proc. Natl. Acad. Sci. USA*, 75 volume, 1929 (1978).

For example, transformation of an insect or insect cell can be carried out according to method in accordance with *Bio/Technology*, 6, 47-55 (1988).

For example, transformation of animal cell can be carried out according to methods described in cell technology supplement volume 8, new cell technology test protocol, method 263-267 (1995) (published by Shujun Corp.), Virology, 52 volume, 456 (1973).

In this way transformant transformed with the expression vector which contained DNA which coded G protein-coupled receptor protein of this invention is obtained.

When culturing the transformant wherein the host is Escherichia genus bacteria, Bacillus genus bacteria, liquid medium is suitable as culture medium used, and source of carbon, nitrogen source, inorganics others which are necessary for growth of said transformant are contained in the culture therein. For example, as source of carbon, glucose, dextrin, soluble starch, sucrose are proposed, for example, as nitrogen source, ammonium salt species, nitrate species corn steep liquor, peptone, casein, meat extract, soybean cake, inorganic or organic substance such as the Solanum tuberosum extract are proposed, for example, as inorganics, calcium chloride, sodium dihydrogenphosphate, magnesium chloride are nominated. Moreover yeast, vitamin species, growth accelerator may be added to. As for pH of culture medium, about 5-8 are desirable.

As culture medium of when cultured Escherichia genus bacteria, for example, M9 culture medium [Milier, Journal of Experiments in Molecular Genetics, 431, 433, Cold Spring Harbor Laboratory, New York 1972] containing glucose, casamino acid is preferred. For example, drug such as 3 - indolyl acrylic acid can be added thereto in accordance with requirements in order to cause promoter to function efficiently. When the host is Escherichia genus bacteria, culturing is carried out for about 3-24 hours at usually about 15-43 degrees and in accordance with requirements it can be aerated and stirred.

When the host is Bacillus genus bacteria, the culturing is carried out for about 6-24 hours at usually about 30-40 degrees and in accordance with requirements it can be aerated and stirred.

When culturing the transformant wherein the host is yeast, as culture medium, for example Burkholder minimal media [Bostian K.L. et al. Proc. Natl. Acad. Sci. USA, 77 volume, 4505 (1980)] and SD culture medium containing 0.5 % casamino acid [Bitter, G. A. et al. Proc. Natl. Acad. Sci. USA, 81, 5330 (1984)] are nominated. It is preferred that pH of culture medium is adjusted to

about 5-8. Culturing is carried out for about 24-72 hours at usually about 20 degrees-35 degrees and in accordance with requirements it is aerated and stirred.

When the transformant that host is an insect or insect cell is cultured, as culture medium, the ones which were suitably added additives of for example 10 % bovine serum inactivated in Grace's Insect Medium (Grace, T. C. C., Nature, 195, 788 (1962)) are used. It is preferred that about pH of culture medium is adjusted to 6.2-6.4. Culturing is carried out at usually about 27 degrees for about 3-5 day periods and in accordance with requirements it is aerated and stirred.

When culturing the transformant wherein the host is animal cell, as culture medium, for example, MEM culture medium [Science, 122 volume, 501 (1952)], containing about 5-20 % foetal bovine serums, DMEM culture medium [Virology, 8 volume, 396 (1959)], RPMI 1640 culture medium [The Journal of the American Medical Association, 199 volume, 519 (1967)], 199 culture medium [Proceedings of the Society for the Biological Medicine, 73 volume, 1 (1950)] are used. As for pH, it is preferred to be about 6-8. Culturing is carried out for about 15-60 hours at usually about 30 degrees to 40 degrees and in accordance with requirements it is aerated and stirred.

As described above, the G protein-coupled receptor protein of this invention or partial peptide thereof can be formed on the cell membrane of the transformant.

For example, the separation and purification of the receptor protein of this invention or partial peptide thereof can be carried out by the following method from aforesaid cultured material.

When receptor protein of this invention or partial peptides thereof are extracted from culture body cells or cells, method wherein after culturing, body cells or cells are collected by well known method, and these are suspended in suitable buffer solution, body cells or cells are destroyed by supersonic wave, freeze-thawing and/or lysozyme, and next centrifugal separation or filtration is carried out to obtain crude isolated liquid of receptor protein of this invention or partial peptides thereof, is suitably used. Protein denaturant such as urea and guanidine hydrochloride and surface active agent such as triton X-100 [TM] may be included in buffer solution. When receptor protein of this invention or partial peptides thereof are secreted in culture medium, after completion of the culturing it is separated into it with body cells or cell and supernatant liquid with it itself familiar process, and supernatant liquid is recovered.

Purification of receptor protein of this invention to contain in liquid the culture supernatant liquid which was in this way obtained or extraction or partial peptides thereof can be carried out by adequately combining itself well-known separation and purification methods. As these well known separation and purification method, method using solubility such as the salting out and solvent sedimentation method, dialyses method, ultrafiltration process, method mainly using a difference of molecular weight such as SDS-polyacrylamide gel electrophoresis method and Gel filtration, method using a difference of electric charge such as ion exchange chromatography, method using specific affinity such as affinity chromatography, method using a difference of hydrophobic property such as reversed phase high performance liquid chromatography, method using a difference of isoelectric point such as electrophoresis of isoelectric point are used.

When receptor protein of this invention to be obtained or partial peptides thereof is obtained as educt, it can be converted into salt by method of itself well known or method based on this, and conversely it can be converted into other salt or educt by itself familiar process or method based on this in case obtained as salt.

Moreover by applying suitable protein modification enzyme on receptor protein of this invention or partial peptide thereof that is produced by the recombinant body before or after the purification, thereby modification is arbitrarily added, or polypeptide can be eliminated partially. As protein modification enzyme, for example, trypsin, chymotrypsin, arginyl endopeptidase, protein kinase, glycosidase are used.

The activity of receptor protein of this invention or salts thereof, partial peptide or ester thereof or amide thereof of this invention or salts thereof formed in this way can be measured by the enzyme immunoassay using specific antibody and binding experiment with labelled ligand.

Antibody with respect to receptor protein of this invention or partial peptide thereof or salts thereof can be any of polyclonal antibody and monoclonal antibody so long as it is the antibody which can recognize receptor proteins of this invention or partial peptide thereof or salts thereof.

Antibody with respect to receptor proteins of this invention or partial peptide thereof or salts thereof (below there are cases to be abbreviated receptor proteins of this invention) can be

produced using receptor proteins of this invention or partial peptide thereof or salts thereof as an antigen according to a process for the production of antiserum or antibody of itself well-known.

Production of monoclonal antibody

[a] Manufacture of monoclonal antibody-forming cell

The receptor proteins of this invention is administered by itself or with a carrier or diluent to mammalian organisms to the site that antibody production is possible by administration. Complete Freund's adjuvant and incomplete Freund's adjuvant may be administered in order to raise antibody production ability during administration. As for the administration, it is carried out usually once every 2-6 days, 2-10 times in total. As mammalian organisms, for example monkey, rabbit, dog, guinea pig, mouse, rat, sheep, goat are nominated, but rat and mouse are preferably used.

For the manufacture of monoclonal antibody production cell, the body in which antibody titer was observed is selected from the warm-blooded animal for example a mouse, which was immunised with an antigen, and lymph node or the spleen is collected 2-5 days after the final immunisation and it can be prepared as monoclonal antibody production hybridoma by letting antibody-forming cell fuse with myeloma cell. For example, measurement of antibody titer in antiserum can be carried out by measuring the activity of the labelling agent which bonded with the antibody after reacting the antiserum with labelled receptor proteins of this invention or ligand peptide of this invention mentioned later. Fusion operation can be carried out according to already known method, method for example of Kayler and Milstein (Nature), 256, 495 pages (1975). As fusion accelerator, for example polyethylene glycol (PEG) or Sendai virus are proposed, but preferably PEG is used.

As myeloma cell, for example NS-1, P3U1, SP2/0 are nominated, but P3U1 is preferably used. Preferred proportion of used antibody-forming cell (spleen cell) number and myeloma cell number is around 1:1 - 20:1, and PEG (preferably PEG 1000 - PEG 6000) is added preferably by concentration of around 10-80 %, and cell fusion can be efficiently carried out by preferably incubating at about 30-37 degrees for about 1-10 minutes.

Various kinds of methods can be used in screening of monoclonal antibody production hybridoma, but for example, method in which hybridoma culture supernatant liquid is added to the

solid phase (example, micro-plate) on which antigen or receptor proteins was absorbed directly or with a carrier, thereafter method to detect the monoclonal antibody in which protein A or anti-immunoglobulin antibody labelled with radioactive material or enzyme (antimouse immunoglobulin antibody is used in case of mouse cell used in cell fusion) is added, and bonded with the solid phase, method in which hybridoma culture supernatant liquid is added to the solid phase which absorbed protein A or anti-immunoglobulin antibody, and receptor proteins of this invention labelled with radioactive material or enzyme or ligand peptide of this invention is added, and the monoclonal antibody which bonded with the solid phase is detected, are proposed.

Selection of monoclonal antibody can be carried out according to itself well-known method or a method based on this, but can be carried out with culture medium for animal cell added to HAT (hypoxanthine, aminopterin, thymidine) usually. As culturing and selection medium, any kind of culture medium can be used so long as hybridoma can grow. For example, it is possible to use 1-20 %, RPMI 1640 culture medium including bovine foetal serum of preferably 10-20 %, serum-free medium for hybridoma culture (SFM-101 NISSUI Seiyaku Co.), GIT culture medium (Wako Jyunyaku Kogyo Co.) including bovine foetal serum of 1-10 %. Culture temperature is preferably about 37 degrees usually 20-40 degrees. Culture time is preferably from 1 week to 2 weeks, usually from 5 days to 3 weeks. Culture can be carried out usually under 5% carbon dioxide gas. Antibody titer of hybridoma culture supernatant liquid can be measured in the same way as in measurement of antibody titer in aforesaid antiserum.

[b] Purification of monoclonal antibody

In the same way as in separation and refinement of polyclonal antibody, it can be carried out according to ordinary separation and refinement method of immunoglobulin, [example a method of salting out, alcohol sedimentation, isoelectric point sedimentation, electrophoresis, adsorption-desorption method due to ion exchanger (example, DEAE), ultracentrifugal method, gel filtration, antigen bond solid phase method or specific purification method wherein only antibody is gathered by active adsorbate such as protein A or protein G and antibody is obtained by releasing the binding].

Manufacture of polyclonal antibody

Polyclonal antibody of this invention can be produced according to it itself well known method or method based on this. For example, complex of immunity antigen (receptor proteins antigen) and

carrier protein are made and immunisation is carried out with mammalian organisms in the same way as in a process for the production of aforesaid monoclonal antibody, antibody containing material with respect to receptor proteins of this invention is gathered from said immunised animal, and separation and refinement of antibody can be carried out.

On complex of immunity antigen and carrier protein which are used in order to immunise mammalian organisms, the species of carrier and mixing proportion of carrier and hapten can be any type and be crosslinked by any ratio so long as it can efficiently form antibody with respect to hapten immunised by crosslinking to carrier, and for example, method to cause coupling bovine serum albumin, bovine thyroglobulin, keyhole limpet hemocyanin weight ratio with respect to 1 hapten at about 0.1-20, preferably about 1-5.

Moreover for coupling of hapten and carrier, various kinds of condensing agent can be used, but active ester agent containing glutaraldehyde and carbodiimide, maleimide active ester, thiol group, dithiopyridyl group is used.

Condensation product is administered by itself or with a carrier, diluent to the site where antibody production with respect to a warm-blooded animal is possible. Complete Freund's adjuvant and incomplete Freund's adjuvant may be administered in order to raise antibody production ability during administration. Administration can be carried out usually once every about 2-6 weeks, around 3-10 times in total.

Polyclonal antibody can be collected from blood or ascites fluid, preferably blood of mammalian organisms immunised by aforesaid method.

Measurement of polyclonal antibody value in antiserum can be measured in the same way as in measurement of antibody titer in aforesaid bovine serum. Separation and refinement of polyclonal antibody can be carried out according to separation refining method of immunoglobulin same as in separation and refinement of aforesaid monoclonal antibody.

Receptor protein of this invention or salts thereof, partial peptides thereof or salts thereof and DNA coding for said receptor protein or partial peptide thereof can be used for [1] Determination of ligand (agonist) with respect to G protein-coupled receptor protein of this invention, [2]

Preventative and/or therapeutic agent of disease related to dysfunction of G protein-coupled receptor protein of this invention, [3] Genetic screening agent, [4] Screening of a compound that changes the expression of receptor protein of this invention or partial peptides thereof, [5] Preventative and/or therapeutic agent of various illness which contains a compound that changes the expression of receptor protein of this invention or partial peptides thereof, [6] Determination of ligand with respect to G protein-coupled receptor protein of this invention, [7] Screening of the compound (agonist, antagonist) which changes the binding of G protein-coupled receptor protein of this invention and ligand, [8] Preventative and/or therapeutic agent of various illness which contains the compound (agonist, antagonist) which changes the binding of G protein-coupled receptor protein of this invention and ligand, [9] Determination of receptor protein of this invention partial peptides thereof or salts thereof, [10] Screening method of compound that changes the quantity of receptor protein of this invention or partial peptides thereof or salts thereof on cell membrane, [11] Preventative and/or therapeutic agent of various illness which contains the compound that changes the quantity of receptor protein of this invention or partial peptides thereof or salts thereof on cell membrane, [12] Neutralisation by antibody with respect to receptor proteins of this invention or partial peptides thereof or salts thereof, [13] Production of non-human animal having DNA coding for G protein-coupled receptor proteins of this invention.

In particular by using receptor binding assay system that uses expression system of recombinant G protein-coupled receptor protein of this invention, it is possible to screen the compound that changes the binding of ligand with respect to G protein-coupled receptor protein of this invention specific for human and mammalian organisms (example, agonist, antagonist), and said agonist or antagonist can be used as preventative and therapeutic agents of various illness.

Below the use of receptor proteins of this invention or partial peptides thereof or salts thereof (hereafter in some cases abbreviated as receptor proteins of this invention), DNA coding for receptor proteins of this invention or partial peptide thereof (hereafter in some cases abbreviated as DNA of this invention) and antibody with respect to receptor proteins of this invention (hereafter in some cases abbreviated as antibody of this invention) are described with embodiment.

[1] Determination of ligand with respect to G protein-coupled receptor protein of this invention (agonist)

Receptor proteins of this invention or salts thereof, or partial peptide thereof or salts thereof is useful as agent for searching and determining the ligand with respect to receptor protein of this invention or salts thereof (agonist).

In other words this invention puts forward a determination method of ligand with respect to receptor protein of this invention to be characterised in that receptor proteins of this invention or salts thereof, or partial peptide thereof or salts thereof is contacted with test compounds.

As test compounds, well known ligands (for example angiotensin, bombesin, cannabinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal polypeptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related peptide), leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenalin, and -chemokine (chemokine) [for example IL-8, GRO β , GRO γ , GRO δ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1 α , MIP-1 β , RANTES], endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptide, gallanin) and in addition for example, tissue extract or cell culture supernatant liquid of human or mammalian organisms (for example a mouse, a rat, a pig, bovine, a sheep, a monkey) are used. For example, said tissue extract or cell culture supernatant liquid is added to receptor proteins of this invention, fractionation is carried out while measuring the cell stimulation activities and finally a single ligand can be obtained.

As embodiments, the ligand determination method of this invention is a method in which by using receptor proteins of this invention or partial peptide thereof or salts thereof, or building expression system of recombinant receptor protein and using the receptor binding assay system which uses said expression system, a compound (for example peptide, protein, non-peptide compounds, synthetic compound, fermentation product) or salts thereof having a cell stimulation activity by binding to the receptor protein of this invention (the activity for example, to inhibit or the activity to promote arachidonic acid release, acetylcholine release, intracellular Ca²⁺ release, intracellular cGMP formation, inositol phosphate production, intracellular cAMP formation, cell

membrane potential fluctuation, phosphorylation of intracellular protein, c-fos activation and lowering of pH) is determined.

In ligand determination method of this invention, it is characterised in that when the receptor protein of this invention and test compound are contacted, the binding quantity of test compound with respect to for example said receptor protein or the cell stimulation activity is measured.

More as examples, this invention puts forward,

(1) Determination method of ligand with respect to receptor protein of this invention or salts thereof to be characterised in that when labelled test compound is contacted with receptor proteins of this invention or salts thereof or partial peptide thereof or salts thereof, a quantity of binding of labelled test compound with respect to said proteins or salts thereof or partial peptide thereof or salts thereof is measured,

(2) Determination method of ligand with respect to receptor protein of this invention to be characterised in that when labelled test compound is contacted in the membrane fraction of said cell or the cell which contained receptor protein of this invention, the binding quantity of labelled test compound with respect to the said membrane fraction or said cell or salts thereof is measured,

(3) Determination method of ligand with respect to receptor protein of this invention characterised in that when labelled test compound is contacted with receptor protein expressed on cell membrane by culturing the transformant containing DNA coding for receptor protein of this invention, the binding quantity of labelled test compound with respect to said receptor proteins or salts thereof is measured,

(4) Determination method of ligand with respect to receptor protein of this invention or salts thereof to be characterised in that when test compound is contacted with the cell which contains receptor proteins of this invention, the cell stimulation activity through receptor protein (the activity for example, to inhibit or the activity to promote arachidonic acid release, acetylcholine release, Ca release in cell, intracellular cGMP formation, inositol phosphate production, intracellular cAMP formation, cell membrane potential fluctuation, phosphorylation of intracellular protein, c-fos activation and lowering of pH) is measured, and

(5) Determination method of ligand with respect to receptor protein of this invention or salts thereof to be characterised in that when test compound is contacted with receptor protein expressed on cell membrane by culturing the transformant containing DNA coding for receptor

protein of this invention, the cell stimulation activity through receptor protein (the activity for example, to inhibit or the activity to promote arachidonic acid release, acetylcholine release, Ca release in cell, intracellular cGMP formation, inositol phosphate production, intracellular cAMP formation, cell membrane potential fluctuation, phosphorylation of intracellular protein, c-fos activation and lowering of pH) is measured.

In particular an examination of aforesaid (1)-(3) is carried out, and that test compound is confirmed to bind to receptor proteins of this invention, and thereafter examination of aforesaid (4)-(5) is preferably carried out.

At first, as receptor proteins of this invention to be used in ligand determination method, anything can be used so long as it is aforesaid receptor protein of this invention or the one containing partial peptide of this invention, but the receptor protein of this invention that is expressed in mass using animal cell are suitable for it.

The above-mentioned expression method is used to produce receptor protein of this invention, but it is preferred to be carried out by expressing DNA coding for said receptor proteins with mammalian organisms cell and insect cell. In DNA fragment coding target protein section, complementary DNA is used usually, but is not always limited to this. For example, gene fragment and synthetic DNA may be used. In order to introduce DNA fragment coding for receptor protein of this invention is introduced into host animal cell, and to efficiently express them, it is preferred that said DNA fragment is incorporated downstream of such as polyhedrin promoter of nuclear polyhedrosis virus (nuclear polyhedrosis virus; NPV) belonging to baculovirus with insect as a host, promoter derived from SV40, promoter of a retrovirus, metallothionein promoter, human heat shock promoter, cytomegalovirus promoter, SR promoter. The test of quantity and quality of the receptor which appeared can be carried out with it itself familiar process. For example, it can be carried out according to method in the literature [Nambi, P. et al. The journal of biological chemistry, vol. 267, pp. 19555-19559, 1992].

Accordingly, in ligand determination method of this invention, the one containing receptor proteins of this invention or partial peptide thereof or salts thereof may be the receptor protein which was refined according to it itself familiar process, or cell containing said receptor protein or the cell membrane fraction thereof.

In ligand determination method of this invention, when cell containing receptor protein of this invention is used, said cell may be immobilised with glutaraldehyde, formalin. Immobilisation method can be carried out according to itself familiar process.

As cell containing receptor proteins of this invention, the host cell which expressed receptor protein of this invention is referred to, and as said host cell, E. Coli, Bacillus subtilis, yeast, insect cell, animal cell are used.

As the cell membrane fraction, cell is pulverised thereafter, a fraction containing most cell membrane which is obtained by itself familiar process is referred to. As method of pulverisation of cell, method to crush cell with Potter-Elvehjem type homogeniser, pulverisation with Waring blender and polytron (made by Kinematica Co.), pulverisation by supersonic wave, pulverisation by jetting cell from thin nozzle is nominated while pressurising it with French press are nominated. In fractionation of cell membrane, differential centrifugation method and fractionation by centrifugal force such as density-gradient centrifugation are mainly used. For example, cell breaking liquid is centrifuged at low speed (500 rpm -3000 rpm) for a short time (for usually 1-10 minutes), and supernatant liquid is centrifuged for higher speed (15000 rpm -30000 rpm) usually from 30 minutes to 2 hours, and the obtained sedimentation is regarded as the membrane fraction. In the said membrane fraction, receptor protein and phospholipid derived from the cell and membrane constituent such as membrane protein are contained much.

It is suitable that cell containing said receptor protein and the quantity of receptor protein in the membrane fraction thereof are for example 10 to the power 3 to 10 to the power 8 molecules per cell, it is preferred 10 to the power 5 to 10 to the power 7 molecules. Moreover ligand avidity (specific activity) per membrane fraction becomes high with many quantities of expression, and assembly of the screening system with high sensitivity becomes possible, and a large quantity of sample can be measured with the same lot.

In order to carry out method of aforesaid (1)-(3) for determining ligand with respect to receptor protein of this invention or salts thereof, the suitable receptor protein fraction and labelled test compound are necessary.

As the receptor protein fraction, the natural receptor protein fraction or the recombinant receptor fraction with the same activity as this is desirable. Wherein the equal activity shows equal ligand avidity, signal transduction action.

As labelled test compounds, angiotensin, bombesin, cannabinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, an opioid, purine, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal poly peptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related peptide), leukotriene, prostaglandin, thromboxane, adenosine, adrenalin, and α -chemokine (for example IL-8, GRO β , GRO γ , GRO δ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP1 β , RANTES), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptide, the gallanin which were labelled with [3H], [125 I], [14C], [35S], are suitable.

As embodiments, in order to carry out determination method of ligand with respect to receptor protein of this invention, first cell or membrane fraction of cell containing receptor protein of this invention is suspended in buffer suitable for the determination method, and thereby receptor preparation is prepared. As buffer, it can be any buffer so long as it is the buffer which do not hinder the binding of ligand and receptor proteins such as phosphoric acid buffer of pH 4-10 (preferably pH 6-8), tris-hydrochloric acid buffer. Moreover, for the purpose of reducing non-specific binding, surface active agent such as CHAPS, Tween-80 [TM] (Kao-Atlas Co.) digitonin, deoxycholate and various protein such as bovine serum albumin and gelatine can be added to the buffer. Furthermore, for the purpose of suppressing decomposition of receptor and ligand by protease, protease inhibitor such as PMSF, leupeptin, E-64 (Peptide Kenkyusho), pepstatin can be added to. Test compound labelled with [3H], [125I], [14C], [35S] of fixed quantity (5000 cpm-500000 cpm) is caused to be copresent in 0.01 ml-10 ml said receptor solution. In order to know the quantity of non-specific binding (NSB) the reaction tube is prepared to which was added unlabelled test compound in large excess. Reaction is carried out from about 0 degrees 50 degrees, preferably from about 4 degrees to 37 degrees between about 20 minutes - 24 hours, or preferably between about 30 minutes - 24 hours. On completion of the reaction it is filtered with glass fibre filter paper and is washed with same buffer of suitable quantity, next the radiation activity to remain in glass fibre filter paper is measured with liquid scintillation counter or β -counter. The test compound in which the count (B-NSB) subtracting quantity of non-specific

binding (NSB) from quantity of total binding (B) exceeds 0 cpm can be selected as ligand with respect to receptor protein of this invention or salts thereof (agonist).

In order to carry out method of aforesaid (4)-(5) for determining ligand with respect to receptor protein of this invention or salts thereof, the cell stimulation activities through said receptor protein, (the activity for example, to inhibit or the activity to promote arachidonic acid release, acetylcholine release, intracellular Ca²⁺ release, intracellular cGMP formation, inositol phosphate production, intracellular cAMP formation, cell membrane potential fluctuation, phosphorylation of intracellular protein, c-fos activation and lowering of pH) can be measured using a kit for commercial measurement or well known method. As embodiments first the cell which contains receptor protein is cultured in multiwell plates. When ligand determination is carried out, it is changed to fresh culture medium or the suitable buffer which does not show toxicity to cell beforehand, test compounds are added to and are incubated for fixed time, and next cell is extracted or supernatant liquid is recovered and formed product is determined according to each method. When the assay of formation of substance comprising an index of cell stimulation activity (for example arachidonic acid) is difficult due to the degrading enzyme contained in cells, inhibitor with respect to said degrading enzyme is added to, and assay may be carried out. Moreover, about the activity such as cAMP production inhibition, it can be detected as production inhibitory action with respect to cell increased the fundamental production quantity of cell with forskolin.

A determination kit for ligand that binds to receptor protein of this invention or salts thereof contains receptor protein of this invention or salts thereof, partial peptide thereof or salts thereof, the membrane fractions of cell containing receptor proteins of this invention or cell containing receptor proteins of this invention.

As example of a kit for ligand determination of this invention, the followings are proposed.

1 Agent for ligand determination

(1) Buffer for washing and buffer used for measurement

Hanks' Balanced Salt Solution (made by Gibco Co.), added with bovine serum albumin of 0.05 % (made by Sigma Co.).

It is filtered and sterilised with filter of pore size 0.45 μm , and stored at 4 degrees or may be prepared at the time of use.

(2) G protein-coupled receptor protein preparation

CHO cell which expressed receptor protein of this invention is subcultured with 5×10^5 to the power 5/ well in 12-well plate and the one which was cultured at 37 degrees, 5% CO₂, 95% air for two days.

(3) Labelled test compound

The one which was labelled by suitable method or the compound labelled with commercial [³H], [¹²⁵I], [¹⁴C], [³⁵S].

Aqueous solution is stored at 4 degrees or -20 degrees, and it is diluted to 1 μM with buffer for measurement at the time of use. As for test compound showing poor solubility to water, it is dissolved in dimethylformamide, DMSO, methanol.

(4) Non-label test compound

It is prepared 100-1000 times strong concentration in one same as labelled compound.

2. Measuring method

(1) Receptor protein expression CHO cell of this invention cultured using 12 well-plate for tissue culture is washed twice with 1 ml buffer used for measurement, and next buffer used for measurement of 490 μl is added in each well.

(2) Labelled test compound 5 μl is added and is reacted at room temperature for one hour. In order to know the non-specific binding quantity, non-label test compound 5 μl is added.

(3) The reaction liquor is eliminated and is washed three times with 1 ml buffer for washing. Labelled test compound bound to cell is dissolved in 0.2 N NaOH-1% SDS, and it is mixed with 4 ml liquid scintillator A (made by Wako Jyunyaku).

(4) Liquid scintillation counter (Beckman Co.) is used, and the radiation activity is measured.

For example, as the ligand which can bind to receptor protein of this invention or salts thereof, the substances which are specifically present in brain, hypophysis, pancreas are proposed, as embodiments, angiotensin, bombesin, cannabinoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal and related polypeptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related peptide), leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenalin, and chemokine (chemokine) (for example IL-8, GRO β , GRO γ , GRO δ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptide, gallanin, are used.

[2] Preventative and/or therapeutic agent of disease related to dysfunction of G protein-coupled receptor protein of this invention

In the method of [1], once the ligand with respect to receptor protein of this invention becomes clear, (1) receptor proteins of this invention or (2) DNA coding for said receptor proteins can be used as preventative and/or therapeutic agent of disease related to dysfunction of receptor protein of this invention according to the action of said ligand.

For example, when a patient whose physiological effect of ligand is not expected because receptor protein of this invention is decreased in living matter (a deficiency disease of said receptor protein) exists, (1) receptor protein of this invention are administered to a said patient, and the quantity of for example said receptor protein is supplemented, (2) [a] by administering to a said patient, and expressing DNA coding for receptor protein of this invention, or [b] by inserting DNA coding for receptor proteins of this invention to targeted cell, and causing to express, and next transplanting said cell to said patient, the quantity of receptor protein of this invention inside of the body of a patient is increased and thereby the action of ligand can be fully attained. In other words DNA coding for receptor protein of this invention is useful as medicine such as preventative and/or therapeutic agent of disease related to dysfunction of receptor protein of this invention that is safe and low toxicity.

The receptor protein of this invention is found to have about 30 % homology in amino acid level to MAS which is one species of G protein-coupled receptor protein. Because there is a report that

a change of central function such as accentuation of anxiety was found in MAS gene deficient mouse [J. B. C., 273, (No. 19), 11867-11873 (1998)], the MAS gene is thought to have some role in the expression of central function. Accordingly, the receptor protein of this invention in which homology to MAS is found, is useful for prevention and/or treatment of diseases related to dysfunction of central function (for example, insanity including anxiety, schizophrenia, cyclic psychosis, dementia, mental deficiency and movement disorder, and the like). Because, there is a report that the expression of rat MAS gene showed high expression in various peripheral organs immediately after birth, and the high expression was observed in testis other than central system after maturation, it is thought to have an important role in the cell proliferation • acquisition of function and reproduction. Accordingly, the receptor protein of this invention in which homology to MAS is found, is useful for prevention and/or treatment of respiratory diseases, cardiovascular diseases, gastrointestinal diseases, liver / cholecyst / pancreas diseases, endocrine diseases.

When the receptor proteins of this invention is used as aforesaid preventative and therapeutic agent, it can be formulated pharmaceutically according to usual practice.

On the other hand when DNA coding for receptor proteins of this invention (below there are case to be abbreviated as DNA of this invention) is used as aforesaid preventative and therapeutic agent, is used alone or after inserted in suitable vector such as retrovirus vector, adeno virus vector, adeno virus associated virus vector, next it can be carried out according to usual practice. The DNA of this invention can be administered without further treating or together with adjuvant for intake promotion by catheter such as gene gun and hydrogel catheter.

For example, (1) receptor proteins of this invention or (2) DNA coding for said receptor proteins of this invention can be used orally as tablet, capsule agent, elixir medicine, microcapsule medicine which sugar coating was made in accordance with requirements, or orally in the form of injection such as suspension or sterile solution of water and pharmacologically permitted liquid other than water. For example, (1) receptor proteins of this invention or (2) DNA coding for said receptor proteins of this invention can be produced by being admixed into the unit dosage form that the formulation which is generally required with a pharmacologically recognised well known carrier, flavouring agent, excipient, vehicle, antiseptic, stabiliser, binder. As for the quantity of effective ingredient at this formulation, suitable capacity in the indicated range can be obtained.

[0060]

As the additive which can be admixed into a tablet, capsule agent, for example constituents such as gelatine, corn starch, tragacanth, gum Arabic, excipient such as crystalline cellulose, swelling agent such as corn starch, gelatine, alginic acid, lubricant such as magnesium stearate, flavouring agents such as a cherry, peppermint, akamono oil, sweetener such as saccharin or sucrose, milk sugar were used. When dispensing unit form is capsule, in addition to material of aforesaid type, liquid state carrier such as oils and fats can be contained. A sterile composition for injection can be prescribed according to the ordinary formulation in which the active material and the naturally produced vegetable oil such as sesame oil, coconut in the vehicle such as water used for injection are suspended or dissolved. As aqueous liquid for injection, for example, isotonic liquid (for example D-sorbitol, D-mannitol, sodium chloride) including physiological saline, glucose and other supporting drug is used, and suitable solubiliser, for example alcohol (example, ethanol), polyalcohol (example, propylene glycol, polyethylene glycol), nonionic detergent (example, polysorbate 80 [TM], HCO-50) may be used together. As oily liquid, for example, sesame oil, soya bean oil are used, it may be used together with solubiliser such as benzyl benzoate, the benzyl alcohol.

[0061]

Moreover aforesaid preventative and therapeutic agent can be formulated with for example buffer (for example phosphate buffer solution, sodium acetate buffer solution), analgesic (for example benzalkonium chloride, procaine hydrochloride), stabiliser (for example human serum albumin, polyethylene glycol), storage agent (for example benzyl alcohol, phenol), oxidation inhibitor. The injection which was prepared is packed into usually suitable ampoule.

Because drug obtained in this way is safe and low toxicity, can be administered for example to human and mammalian organisms (for example rat, rabbit, sheep, pig, bovine, cat, dog, monkey).

As for the dosage of for example receptor proteins of this invention, there is a difference depending on administration subject, target organ, symptoms, administration method, but in case of oral administration, for example, generally in schizophrenia patients (as 60 kg), it is about 0.1 mg-100 mg per day and is preferably about 1.0-50 mg and is more preferably about 1.0-20 mg. When it is orally administered, dose per time thereof is different depending on administration

subject, target organ, symptoms, administration method, but it is about around 0.01-30 mg per day in usually for example in schizophrenia patient (as 60 kg) in form of injection and is preferably about around 0.1-20 mg, and is more preferably about around 0.1-10 mg and administration using an intravenous injection is convenient. In case of other animal, the quantity calculated as per 60 kg can be administered.

As for the dosage of DNA coding for the receptor proteins of this invention there is a difference depending on administration subject, target organ, symptoms, administration method, but in case of oral administration, for example, in schizophrenia patient (as 60 kg), it is about 0.1 mg-100 mg per day and is preferably about 1.0-50 mg and is more preferably about 1.0-20 mg generally. When it is orally administered, dose per time thereof is different depending on administration subject, target organ, symptoms, administration method, but it is about around 0.01-30 mg per day in usually for example in schizophrenia patient (as 60 kg) in form of injection and is preferably about around 0.1-20 mg, and is more preferably about around 0.1-10 mg and administration using an intravenous injection is convenient. In case of other animal, the quantity calculated as per 60 kg can be administered.

[3] Genetic screening agent

Because by using DNA of this invention as a probe, anomaly of mRNA (gene anomaly) or DNA which codes for receptor peptide of this invention or partial peptide thereof can be detected in mammalian organisms (for example rat, a rabbit, a sheep, a pig, bovine, a cat, a dog, a monkey) or human, for example, it is useful as genetic screening medicine such as increase or over-expression of mRNA or said DNA, mutation or expression lowering of mRNA or said DNA.

Aforesaid genetic screening using DNA of this invention can be carried out by itself well-known method for example Northern hybridisation and PCR-SSCP method (Genomics, Vol. 5, 874-879 pages (1989), Proceedings of the National Academy of Sciences of the United States of America, Vol. 86, 2766-2770 pages (1989)).

[4] Screening method of the compound that changes the quantity of expression of receptor protein of this invention or partial peptide thereof

By using as a probe, the DNA of this invention can be used for screening the compound that changes the quantity of expression of receptor protein of this invention or partial peptide thereof.

Namely, this invention puts forward, for example, a screening method for the compound that changes the quantity of expression of receptor protein of this invention or partial peptide thereof by measuring mRNA quantity of receptor protein of this invention or partial peptide thereof contained in (i) tissue or cells isolated from (1) blood, (2) specific organ (3) organs of non-human mammalian animals or (ii) transformant.

The measurement of mRNA quantity of receptor protein of this invention or partial peptide thereof is carried out in an embodiment as follows.

(i) Drugs (for example, anti-dementia drug, antihypertensive, anti-tumour agent, anti-obesity agent) or physical stress (for example water immersion stress, electric shock, light-dark, low temperature) is given to normal or disease model non-human mammalian animals (for example rat, mouse, rabbit, sheep, pig, bovine, cat, dog, monkey, more as embodiment, dementia rat, obese mouse, arteriosclerosis rabbit, tumour bearing mouse), a specified period has passed, and next, the tissue or cells isolated from blood or specific organ (for example brain, liver, kidney) or organs is obtained.

The mRNA of receptor protein of this invention or partial peptide thereof contained in the obtained cells can be determined, by extracting mRNA from cells by usual method and by using for example TaqManPCR, and can be analysed by Northern blot by itself well known method.

(ii) Transformant expressing the receptor protein of this invention or partial peptide thereof is produced in accordance with aforesaid method, the mRNA of receptor protein of this invention or partial peptide thereof contained in said transformant can be determined and analysed in the same way.

The screening of the compound that changes the quantity of expression of receptor protein of this invention or partial peptide thereof can be carried out by method,

(i) wherein test compound is administered to the normal or disease model non-human mammalian animals at the same time or before giving drug or physical stress by a specified time (30 minutes

before to 24 hours before, preferably 30 minutes before to 12 hours before, more preferably 1 hour before to 6 hours before) or after a specified period (30 minutes later to 3 days later, preferably 1 hour later to 2 days later, more preferably 1 hour later to 24 hours later), after a specified time has passed after administration (30 minutes later to 3 days later, preferably 1 hour later to 2 days later, more preferably 1 hour later to 24 hours later), the quantity of mRNA of receptor protein of this invention or partial peptide thereof contained in cells is determined and analysed,

(ii) wherein test compound is mixed in the culture medium during culturing of transformant by conventional method, after culturing for a specified time (1 day later to 7 days later, preferably 1 days later to 3 days later, more preferably 2 days later to 3 days later), the quantity of mRNA of receptor protein of this invention or partial peptide thereof contained in said transformant is determined and analysed.

The compound or salts thereof obtained by using screening method of this invention is a compound that has an action of changing the quantity of expression of receptor protein of this invention or partial peptide thereof, and as embodiment, (a) compound that enhances the cell stimulation activity through G protein-coupled receptor protein (for example the activity to inhibit or the activity to promote arachidonic acid release, acetylcholine release, intracellular Ca²⁺ release, intracellular cGMP formation, inositol phosphate production, intracellular cAMP formation, cell membrane potential fluctuation, phosphorylation of intracellular protein, c-fos activation and lowering of pH) by increasing the expression quantity of receptor protein of this invention or partial peptide thereof and (b) compound that decreases said cell stimulation activity by decreasing the expression quantity of receptor protein of this invention or partial peptide thereof.

As said compound, peptide, protein, the non-peptide compound, synthetic compound, fermentation product are nominated and these compounds can be a novel compound or well known compound.

The compound that enhances said cell stimulation activity is useful as medicine which has low toxicity for safely enhancing the physiological activity of receptor proteins of this invention.

The compound that decreases said cell stimulation activity is useful as the medicine which has low toxicity for safely decreasing the physiological activity of receptor proteins of this invention.

When the compound or the salts thereof which can be obtained by using screening process of this invention is used as aforesaid medicinal composition, it can be carried out according to usual practice. For example, in the same way as in aforesaid medicine containing receptor protein of this invention, it can be made into tablet, capsule agent, elixir medicine, microcapsule medicine, sterile solution, suspension.

Because drug obtained in this way is safe and low toxicity, can be administered for example to human and mammalian organisms (for example a rat, a sheep, a pig, bovine, a cat, a dog, a monkey).

As for the dosage of the said compound or salts thereof, there is a difference depending on the administration subject, target organ, symptoms, administration method, but, in case of oral administration, is from about 1.0-50 mg, preferably about 1.0-20 mg, preferably about 0.1-100 mg per day generally for example in schizophrenia patient (as 60 kg). When it is orally administered, dose per time thereof is different depending on administration subject, target organ, symptoms, administration method, but what is administered using an intravenous injection by about around 0.01-30 mg per day usually for example in schizophrenia patient (as 60 kg) in form of for example injection, 0.1-20 mg more preferably about around 0.1-10 mg is preferably convenient. In case of other animal, the quantity calculated as per 60 kg can be administered.

[5] Preventative and/or therapeutic agent for various diseases which contains the compound that changes the quantity of expression of receptor proteins of this invention or partial peptide thereof

The receptor protein of this invention is as mentioned above, thought to play some important role in-vivo for example central function. Accordingly, the compound which changes the quantity of expression of receptor proteins of this invention or partial peptide thereof can be used as preventative and/or therapeutic agent of various diseases related to dysfunction of receptor protein of this invention.

When said compound is used as preventative and/or therapeutic agent of various illness related to dysfunction of receptor protein of this invention, it can be carried out according to usual practice.

For example, the said compound can be used orally as the tablet applied with sugar coating in accordance with requirement, capsule agent, elixir medicine, microcapsule medicine or orally in the form of injection such as suspension or sterile solution of pharmacologically permitted liquid other than water. For example, it can be produced by admixture in the unit dosage form in a formulation with what is generally recognised as being a pharmacologically recognised well known carrier, flavouring agent, excipient, vehicle, antiseptic, stabiliser, binder. As for the quantity of effective ingredient in this formulation, a suitable quantity in a range as indicated by the field is obtained.

As the additive which can be admixed into a tablet, capsule agent, for example constituents such as gelatine, corn starch, tragacanth, gum Arabic, excipient such as crystalline cellulose, swelling agent such as corn starch, gelatine, alginic acid, lubricant such as magnesium stearate, flavouring agents such as a cherry, peppermint, akamono oil, sweetener such as saccharin or sucrose, milk sugar were used. When dispensing unit form is capsule, in addition to material of aforesaid type, liquid state carrier such as oils and fats can be contained. A sterile composition for injection can be prescribed according to the ordinary formulation in which the active material and the naturally produced vegetable oil such as sesame oil, coconut in the vehicle such as water used for injection are suspended or dissolved. As aqueous liquid for injection, for example, isotonic liquid (for example D-sorbitol, D-mannitol, sodium chloride) including physiological saline, glucose and other supporting drug is used, and suitable solubiliser, for example alcohol (example, ethanol), polyalcohol (example, propylene glycol, polyethylene glycol), nonionic detergent (example, polysorbate 80 [TM], HCO-50) may be used together. As oily liquid, for example, sesame oil, soya bean oil are used, it may be used together with solubiliser such as benzyl benzoate, the benzyl alcohol.

Moreover aforesaid preventative and therapeutic agent can be formulated with for example buffer (for example phosphate buffer solution, sodium acetate buffer solution), analgesic (for example benzalkonium chloride, procaine hydrochloride), stabiliser (for example human serum

albumin, polyethylene glycol), storage agent (for example benzyl alcohol, phenol), oxidation inhibitor. The injection which was prepared is packed into usually suitable ampoule.

Because drug obtained in this way is safe and low toxicity, can be administered for example to human and mammalian organisms (for example rat, rabbit, sheep, pig, bovine, cat, dog, monkey).

As for the dosage of for example ligand peptide of this invention, there is a difference depending on administration subject, target organ, symptoms, administration method, but in case of oral administration, for example, in schizophrenia patient (as 60 kg), it is about 0.1 mg-100 mg per day and is preferably about 1.0-50 mg and is more preferably about 1.0-20 mg generally. When it is orally administered, dose per time thereof is different depending on administration subject, target organ, symptoms, administration method, but it is about around 0.01-30 mg per day in usually for example in schizophrenia patient (as 60 kg) in form of injection and is preferably about around 0.1-20 mg, and is more preferably about around 0.1-10 mg and administration using an intravenous injection is convenient. In case of other animal, the quantity calculated as per 60 kg can be administered.

[6] Assay of ligand with respect to G protein-coupled receptor proteins of this invention

Because the receptor proteins of this invention has binding ability with respect to ligand, the ligand concentration in living matter can be determined with high sensitivity.

For example, assay of this invention is possible to use by combining competition method. In other words, ligand concentration in analyte can be measured by contacting analyte with receptor proteins of this invention. As embodiments for example, it is possible to use according to method in accordance with following (1) or (2) or method based on this.

(1) Hiroshi Irie ed. "radioimmunoassay" (published by Kodansha, 1974)

(2) Hiroshi Irie ed. "radioimmunoassay, sequel" (published by Kodansha, 1979)

[7] Screening method of a compound (agonist, antagonist) that changes binding of G protein-coupled receptor protein of this invention or salt thereof and ligand

By using receptor proteins of this invention or by building the expression system of recombinant receptor proteins and using receptor binding assay system using said expression system, a compound (for example peptide, protein, the non-peptide compound, synthetic compound, fermentation product) that changes binding of receptor proteins of this invention and ligand can be screened efficiently.

Such compound includes [a] compounds that have cell stimulation activity through G protein-coupled receptor (for example the activity to inhibit or the activity to promote arachidonic acid release, acetylcholine release, intracellular Ca²⁺ release, intracellular cGMP formation, inositol phosphate production, intracellular cAMP formation, cell membrane potential fluctuation, phosphorylation of intracellular protein, c-fos activation and lowering of pH) (so-called agonist with respect to the receptor proteins of this invention) or [b] compounds which do not have the said cell stimulation activity (so-called antagonist with respect to receptor proteins of this invention) or [c] compounds that enhances the binding power of the ligand and receptor proteins of this invention or [d] compounds that decreases the binding power of the ligand and receptor proteins of this invention (wherein, the compound of aforesaid [a] is preferably screened by the aforesaid ligand determination method).

In other words, this invention puts forward screening process of the compound or salts thereof to change the binding of ligand and receptor proteins of this invention or partial peptide thereof or salts thereof to be characterised in that the case wherein [i] receptor proteins or partial peptide thereof or salts thereof and ligand are contacted and [ii] receptor proteins or partial peptide thereof or salts thereof, ligand and test compound are contacted are compared.

In screening process of this invention, it is characterised in that in case of [i] and [ii], for example, the binding quantity of ligand with respect to said receptor proteins, the cell stimulation activity are measured, and are compared.

More as examples, this invention puts forward,

(1) Screening process of the compound or salts thereof that changes binding of ligand and receptor protein of this invention to be characterised in that a quantity of binding of labelled ligand with respect to said receptor proteins is measured, and is compared when labelled ligand was

contacted with receptor proteins of this invention and when test compound and labelled ligand were contacted with receptor to proteins of this invention,

(2) Screening process of the compound or salts thereof that changes binding of ligand and receptor proteins of this invention to be characterised in that when labelled ligand was contacted with the membrane fraction of said cell or the cell containing receptor proteins of this invention and when labelled ligand and test compound were contacted with the membrane fraction of said cell or the cell containing receptor proteins of this invention, the binding quantity of labelled ligand with respect to said membrane fraction or said cell is measured and compared,

(3) Screening process of the compound or salts thereof that changes binding of ligand and receptor proteins of this invention to be characterised in that when labelled ligand was contacted to the receptor proteins that expressed on cell membrane by culturing the transformant which contained DNA of this invention, and when labelled ligand and test compound were contacted to the receptor proteins which expressed on cell membrane by culturing the transformant which contained DNA of this invention, the binding quantity of labelled ligand with respect to said receptor proteins of this invention is measured and compared,

(4) Screening process of the compound or salts thereof that changes binding of ligand and receptor proteins of this invention to be characterised in that when the compound which activates receptor proteins of this invention (for example ligand with respect to receptor proteins of this invention) was contacted to the cell containing receptor proteins of this invention, and when the test compound and the compound which activates receptor proteins of this invention were contacted to the cell containing receptor proteins of this invention, and cell stimulation activity through the receptor (for example, the activity to inhibit or the activity to promote arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cGMP formation, inositol phosphate production, intracellular cAMP formation, cell membrane potential fluctuation, phosphorylation of intracellular protein, c-fos activation and lowering of pH) is measured and compared, and

(5) Screening processes of the compound or salts thereof that changes binding of ligand and receptor proteins of this invention to be characterised in that when the compound which activates receptor proteins of this invention (for example ligands with respect to receptor proteins of this invention) was contacted with receptor proteins of this invention that expressed on cell membrane by culturing the transformant which contained DNA of this invention, and when test compound and the compound which activates receptor proteins of this invention were contacted with receptor proteins of this invention that expressed on cell membrane by culturing

the transformant which contained DNA of this invention, and cell stimulation activity through the receptor (for example, the activity to inhibit or the activity to promote arachidonic acid release, acetylcholine release, intracellular Ca²⁺ release, intracellular cGMP formation, inositol phosphate production, intracellular cAMP formation, cell membrane potential fluctuation, phosphorylation of intracellular protein, c-fos activation and lowering of pH) is measured and compared.

When screening antagonist or G protein-coupled receptor agonist, before the receptor proteins of this invention were obtained, method to confirm that first a candidate compounds are obtained using cell containing G protein-coupled receptor protein of rat and the like, tissue or cell membrane fraction thereof (primary screening), thereafter a test to confirm whether said candidate compounds inhibited the binding of ligand and G protein-coupled receptor protein or not (secondary screening) was required. But it was difficult that in practice screening antagonist or agonist with respect to target receptor protein because other receptor protein coexisted, too if the cell membrane fraction was used or cell, tissue without further treatment.

However for example, it is possible to screen the compound inhibiting the binding of ligand and G protein-coupled receptor protein by using rat derived receptor proteins of this invention with good efficiency without a need of primary screening. Furthermore it can be simply evaluated that whether the screened chemical agent is agonist or antagonist.

Below an embodiment description of screening process of this invention is formed.

At first as receptor proteins of this invention to be used in screening process of this invention can be any so long as it is one included in receptor proteins of aforesaid this invention, but the cell membrane fraction of organ of mammalian organisms included in receptor proteins of this invention is suitable. But the human derived organs are extremely difficult to obtained, therefore human derived receptor proteins obtained by expressing in large quantity using recombinant are suitable for screening.

The above-mentioned method is used to produce receptor proteins of this invention, but it is preferred to be carried out by expressing DNA of this invention with lactation cell and insect cell. Complementary DNA is used in DNA fragment coding target protein section, but is not necessarily restricted to these. For example, gene fragment and synthetic DNA may be used. It is preferred

that polyhedrin promoter of nuclearpolyhedrosis virus (nuclearpolyhedrosis virus; NPV) belonging to the baculovirus with host comprising an insect, promoter derived from SV40, promoter of a retrovirus, metallothionein promoter, human heat shock promoter, cytomegalovirus promoter, SR promoter incorporate downstream said DNA fragment in order DNA fragment coding receptor protein of this invention is introduced into host animal cell, and to efficiently express them. The quantity of the receptor which appeared and test of quality can be carried out by themselves well-known methods and are able to be carried out according to for example literature [Nambi, P. et al., (J. Biol. Chem), vol. 267, pp. 19555-19559, 1992].

Accordingly, in screening process of this invention, as one containing receptor proteins of this invention, it may be receptor protein purified in accordance with themselves well-known processes, and cell containing said receptor proteins may be used, and moreover the membrane fraction of cell containing said receptor-proteins may be used. When cell containing receptor proteins of this invention is used in screening process of this invention, said cell may be immobilised with glutaraldehyde, formalin. Immobilisation method can be carried out in accordance with themselves familiar processes. As cell containing receptor proteins of this invention, the host cell which expressed said receptor proteins is expressed, but, as said host cell, E. coli, Bacillus subtilis, yeast, insect cell, animal cell are preferred.

As the cell membrane fraction, it means a fraction containing much of the cell membrane after cell is pulverised. As method of pulverisation of cell, pulverisation by jetting cell from thin nozzle while pressurising with French press, pulverisation by supersonic wave method, pulverisation by Waring blender and a thing of polytron (made by Kinematica Co.), pulverisation with Potter-Elvehjem type homogeniser (made by Kinematica Co.) are proposed. Differential centrifugation method and fractionation by centrifugal force such as density-gradient centrifugation are mainly used in fractionation of cell membrane. For example, cell pulverised liquid is centrifuged with (500 rpm -3000 rpm) short time (1-10 minute), furthermore supernatant liquid can be centrifuged at high speed (15000 rpm - 30000 rpm) with usually for from 30 minutes to 2 hours and the obtained sedimentation is regarded as the membrane fraction. In the said membrane fraction, receptor proteins and phospholipid derived from cell and membrane constituent such as membrane protein which appeared contain much.

The quantity of receptor protein in the membrane fraction or cells containing said receptor proteins is preferably 10^3 - 10^8 molecules per cell and ideally 10^5 - 10^7 molecules. Moreover ligand avidity per the membrane fraction with many quantities of expression (specific activity) becomes high, and assembly of the screening system which are high sensitivity becomes possible, and a large quantity of sample gets possible to be measured with same lot.

For example suitable receptor protein fraction and labelled ligand are necessary in order to carry out aforesaid (1)-(3) for screening the compound which changes the binding of ligand and receptor proteins of this invention.

As the receptor protein fraction, the natural receptor protein fraction or the recombinant receptor protein fraction with the same activity as this is desirable. Wherein the equal activity shows equal ligand avidity, signal transduction action.

As labelled ligand, labelled ligand, the labelled ligand analogue compound are used. Ligand labelled with for example [^3H], [^{125}I], [^{14}C], [^{35}S] is used.

As embodiments, in order to carry out screening of the compound which changes the binding of ligand and receptor proteins of this invention, first it is prepared with receptor protein preparation by being suspended in the buffer which was suitable for screening the membrane fraction of cell or the cell which contains receptor proteins of this invention. As buffer, it can be any buffer so long as it is the buffer which do not hinder binding of ligand and receptor proteins such as phosphoric acid buffer of pH 4-10 (preferably pH 6-8), tris-hydrochloric acid buffer. Moreover, with the object to reduce non-specific binding, surface active agent such as CHAPS, Tween-80 [TM] (Kao-Atlas Co.), digitonin, deoxycholate and various protein such as bovine serum albumin and gelatine can be added to the buffer. Furthermore, with the object to suppress decomposition of receptor and ligand by protease, protease inhibitor such as PMSF, leupeptin, E-64 (Peptide Kenkyusho), pepstatin can be added to. The ligand labelled by fixed quantity (5000 cpm - 500000 cpm) is added to 0.01 ml - 10 ml said receptor solution and test compound of 10^{-4} M to 10^{-10} M is simultaneously caused to be co-present. In order to know the quantity of non-specific binding (NSB) the reaction tube is prepared to which was added unlabelled ligand in large excess. Reaction is carried out from about 0 degrees - 50 degrees,

desirably from about 4 degrees to 37 degrees between about 20 minutes - 24 hours, or preferably between about 30 minutes - 3 hours. On completion of the reaction it is filtered with glass fibre filter paper and is washed with same buffer of suitable quantity, next the radiation activity remaining on glass fibre filter paper is measured with liquid scintillation counter or β -counter. The test compound in which the count (B0-NSB) subtracting quantity of non-specific binding (NSB) from the count (B0) where there is no competing substance is regarded 100 %, the test compound for example showing specific binding (B-NSB) becomes 50 % or less can be selected as candidate substance with competitive inhibitory potency.

In order to carry out method of aforesaid (4)-(5) making the compound screening to change ligand and binding of receptor proteins of this invention, for example, it can measure using well known method or commercial kit for measuring the cell stimulation activity through receptor protein (the activity for example, to inhibit or the activity to promote arachidonic acid release, acetylcholine release, intracellular Ca release, intracellular cGMP formation, inositol phosphate production, intracellular cAMP formation, cell membrane potential fluctuation, phosphorylation of intracellular protein, c-fos activation and lowering of pH).

As embodiments, first, the cell which contains receptor proteins of this invention is cultured on multi-well plates. If screening is carried out, and it is changed to fresh culture medium or the suitable buffer which does not show toxicity to cell beforehand and the test compounds are added and it is incubated for a fixed time, and next the cell is extracted or supernatant liquid is recovered and formed product is determined according to each method. When assay of substance comprising an indicator of cell stimulation activity (for example arachidonic acid) is difficult due to the degrading enzyme contained by the cell, inhibitor with respect to said degrading enzyme is added, and the assay may be carried out. Moreover, as for the activity such as cAMP production inhibition, it can be detected as production inhibitory action with respect to cell with increased fundamental production quantity of cell by forskolin.

The cell which expressed suitable receptor protein is necessary when the screening is carried out by measuring the cell stimulation activity. As for the cell strain which expressed the receptor protein of this invention, the cell strain having natural-type receptor proteins of this invention, or cell strain that expressed recombinant receptor protein described above is desirable.

As test compound, for example peptide, protein, non-peptide compounds, synthetic compound, fermentation product, the cell extract, vegetable extract, the animal tissue extract are used, and this compound may be a novel compound or may be a well known compound.

A kit for screening of the compound or salts thereof to change ligand and binding of receptor proteins of this invention is one containing the membrane fraction of cell containing receptor proteins of this invention or cell containing receptor proteins of this invention receptor proteins of this invention.

As example of a kit for screening of this invention, the followings are proposed.

1. Agent for screening

(1) Buffer for washing and buffer used for measurement

Hanks' Balanced Salt Solution (made by Gibco Co.), added with bovine serum albumin of 0.05 % (made by Sigma Co.).

It is filtered and sterilised with filter of pore size 0.45 μm , and stored at 4 degrees or may be prepared at the time of use.

(2) G protein-coupled receptor protein preparation

CHO cell which expressed receptor protein of this invention is subcultured at 5×10^5 to the power 5/ well in 12-well plate and the one which was cultured at 37 degrees, 5% CO₂, 95% air for two days.

(3) Labelled ligand

The ligand which was labelled with commercial [3H], [125I], [14C], [35S].

Aqueous solution is stored at 4 degrees or -20 degrees, and it is diluted with 1 μM at buffer for measurement at the time of use.

(4) Ligand standard solution

Ligand containing 0.1 % bovine serum albumin (made by Sigma Co.) is dissolved in PBS so as to form 1 mM, and stored at -20 degrees.

2. Measuring method

(1) Receptor protein expression CHO cell of this invention cultured using plate for 12-well tissue culture is washed twice with 1 ml buffer for measurement, and next buffer for measurement of 490 μ l is added in each well.

(2) Test compound solution of 10^{-3} to 10^{-10} M, 5 μ l is added, thereafter labelled ligand 5 μ l is added and is reacted at room temperature for one hour. In order to know the non-specific binding quantity, 10^{-3} M ligand 5 μ l is added instead of test compound.

(3) The reaction liquor is eliminated and washing three times with 1 ml buffer for washing is carried out. Labelled ligand bound to cell is dissolved in 0.2 N NaOH-1% SDS, and it is mixed with 4 ml liquid scintillator A (made by Wako Jyunyaku).

(4) Liquid scintillation counter (Beckman Co.) is used, and the radiation activity is measured, and Percent Maximum Binding (PMB) is determined by the following equation.

$$\text{PMB} = [(B - \text{NSB}) / (B_0 - \text{NSB})] \times 100$$

PMB: Percent Maximum Binding

B: Value when the sample is added

NSB: Non-specific Binding

B₀: Maximum binding quantity

The compound or the salts thereof which can be obtained using a kit for screening or screening process of this invention is a compound having action to change ligand and binding of receptor proteins of this invention, as embodiments [a] the compound having the cell stimulation activity (the activity for example, to inhibit or the activity to promote arachidonic acid release, acetylcholine release, intracellular Ca²⁺ release, intracellular cGMP formation, inositol phosphate production, intracellular cAMP formation, cell membrane potential fluctuation, phosphorylation of intracellular protein, c-fos activation and lowering of pH) through G protein-coupled receptor (so-called agonist for receptor protein of this invention), [b] the compound which does not have the said cell stimulation activity (so-called antagonist with respect to receptor protein of this invention), or [c] the compound enhancing binding power of ligand and G protein-coupled

receptor protein of this invention or [d] the compound decreasing binding power of ligand and G protein-coupled receptor protein of this invention.

As the said compound, peptide, protein, non-peptide compounds, synthetic compound, fermentation product are nominated, and this compound may be a novel compound or may be a well known compound.

Agonist with respect to receptor proteins of this invention has an action which is similar to the physiological activity of the ligand with respect to receptor proteins of this invention, and therefore is useful as the medicine which has low toxicity and is safe according to the said ligand activity.

Because antagonist with respect to receptor proteins of this invention can inhibit the physiological activity that ligand with respect to receptor proteins of this invention has, it is useful as the medicine which has low toxicity for safely inhibiting the said ligand activity.

The compound which enhances the binding power between the receptor protein of this invention and the ligand is useful as the medicine which has low toxicity and safely enhances the physiological activity that the ligand has with respect to receptor proteins of this invention.

The compound which decreases the binding power between the receptor protein of this invention and the ligand is useful as the medicine which has low toxicity and safely decreases the physiological activity that the ligand has with respect to receptor proteins of this invention.

When the compound or the salts thereof which can be obtained using a kit for screening or screening process of this invention is used as aforesaid medicinal composition, it can be carried out according to usual practice. For example, in the same way as in medicine containing DNA of aforesaid this invention, it can be made into tablet, capsule agent, elixir medicine, microcapsule medicine, sterile solution, suspension.

Because drug obtained in this way is safe and low toxicity, it can be administered for example to human and mammalian organisms (for example rat, rabbit, sheep, pig, bovine, cat, dog, monkey).

As for the dosage of the said compound or salts thereof, there is a difference depending on the administration subject, target organ, symptoms, administration method, but, in case of oral administration, is generally for example from about 1.0-50 mg, preferably about 1.0-20 mg, preferably about 0.1-100 mg per day in schizophrenia patient (as 60 kg). When it is orally administered, dose per time thereof is different depending on administration subject, target organ, symptoms, administration method, but what is administered using an intravenous injection by about around 0.01-30 mg per day usually for example in schizophrenia patient (as 60 kg) in form of for example injection, 0.1-20 mg more preferably about around 0.1-10 mg is preferably convenient. In case of other animal, the quantity calculated as per 60 kg can be administered.

[8] Preventative and/or therapeutic agent of various illness containing the compound which changes binding of G protein-coupled receptor proteins of this invention and ligand (agonist, antagonist)

Receptor proteins of this invention, as described earlier, are thought to play some important role within the body, for example central function. Accordingly the compound which changes receptor proteins of this invention and binding ligand (agonist, antagonist) can be used as preventative and/or therapeutic agent of disease related to dysfunction of for example receptor protein of this invention.

When the said compound is used as preventative and/or therapeutic agent of disease related to dysfunction of receptor protein of this invention, it can be formulated pharmaceutically according to usual practice.

For example, the said compound can be used orally as the tablet applied with sugar coating in accordance with requirement, capsule agent, elixir medicine, microcapsule medicine or orally in the form of injection such as suspension or sterile solution of pharmacologically permitted liquid other than water. For example, it can be produced by admixture in the unit dosage form in a formulation with what is generally recognised as being a pharmacologically recognised well known carrier, flavouring agent, excipient, vehicle, antiseptic, stabiliser, binder. As for the quantity of effective ingredient in this formulation, a suitable quantity in a range as indicated by the field is obtained.

As the additive which can be admixed into a tablet, capsule agent, for example constituents such as gelatine, corn starch, tragacanth, gum Arabic, excipient such as crystalline cellulose, swelling agent such as corn starch, gelatine, alginic acid, lubricant such as magnesium stearate, flavouring agents such as a cherry, peppermint, akamono oil, sweetener such as saccharin or sucrose, milk sugar were used. When dispensing unit form is capsule, in addition to material of aforesaid type, liquid state carrier such as oils and fats can be contained. A sterile composition for injection can be prescribed according to the ordinary formulation in which the active material and the naturally produced vegetable oil such as sesame oil, coconut in the vehicle such as water used for injection are suspended or dissolved. As aqueous liquid for injection, for example, isotonic liquid (for example D-sorbitol, D-mannitol, sodium chloride) including physiological saline, glucose and other supporting drug is used, and suitable solubiliser, for example alcohol (example, ethanol), polyalcohol (example, propylene glycol, polyethylene glycol), nonionic detergent (example, polysorbate 80 [TM], HCO-50) may be used together. As oily liquid, for example, sesame oil, soya bean oil are used, it may be used together with solubiliser such as benzyl benzoate, the benzyl alcohol.

Moreover aforesaid preventative and therapeutic agent can be formulated with for example buffer (for example phosphate buffer solution, sodium acetate buffer solution), analgesic (for example benzalkonium chloride, procaine hydrochloride), stabiliser (for example human serum albumin, polyethylene glycol), storage agent (for example benzyl alcohol, phenol), oxidation inhibitor. The injection which was prepared is packed into usually suitable ampoule.

Because drug obtained in this way is safe and low toxicity, can be administered for example to human and mammalian organisms (for example rat, rabbit, sheep, pig, bovine, cat, dog, monkey).

As for the dosage of the said compound or salts thereof, there is a difference depending on the administration subject, target organ, symptoms, administration method, but, in case of oral administration, is generally for example about 0.1-100 mg, preferably about 1.0-50 mg, preferably about 1.0-20 mg, per day in schizophrenia patient (as 60 kg). When it is orally administered, dose per time thereof is different depending on administration subject, target organ, symptoms, administration method, but what is administered using an intravenous injection by about 0.01-30 mg, around 0.1-20 mg more preferably around 0.1-10 mg per day is preferably convenient

usually for example in schizophrenia patient (as 60 kg) in form of for example injection. In case of other animal, the quantity calculated as per 60 kg can be administered.

[9] Determination of receptor proteins of this invention or partial peptide thereof or salts thereof

The antibody of this invention can specifically recognise receptor proteins of this invention, and therefore can be used for the determination of receptor proteins of this invention in test liquids, and in particular for determinations based on sandwich immunity measuring methods. In other words, this invention for example puts forward [i] a process for the determination of receptor proteins of this invention in a test liquid, characterised in that an antibody with respect to receptor proteins of this invention is competitively reacted with labelled receptor proteins and a test liquid, and the proportion of labelled receptor proteins bound to the said antibody is measured, and [ii] a process for the determination of receptor proteins in test liquid, characterised in that an antibody of this invention which has been insolubilised on a carrier and antibody labelled antibody of this invention are reacted at the same time or successively, and thereafter the activity of the labelling agent on the insolubilised carrier is measured.

In aforesaid [ii], it is preferred that one of the antibodies is an antibody that recognises N-terminal of receptor proteins of this invention and the other antibody is an antibody which reacts with the C-terminal of the receptor protein of this invention.

In addition to carrying out measurement of the receptor proteins of this invention using a monoclonal antibody with respect to receptor protein of this invention, detection can be carried out by tissue coloring or the like. For this purpose, antibody molecule itself may be used, and moreover F(ab')₂, Fab' or Fab fraction of antibody molecule may be used. Measuring method using antibody with respect to receptor proteins of this invention, is not restricted in particular. If a measurement method is used wherein the quantity of antibody-antigen composite, antigen or antibody corresponding to the antigen quantity in the test liquid (for example quantity of receptor proteins) is detected, this is calculated using a standard curve prepared using a labelled liquid containing an already known quantity of antigen, any measurement method may be used. For example, nephrometry, competition method, sandwich method and immunometric method are suitably used, but the use of a sandwich method described later having sensitivity, and specificity is in particular preferred.

As labelling agent used with measuring method using labelled substance, for example radioactive isotope, enzyme, fluorescent material, light generating substance are used. For example, as radioactive isotope, [125I], [131I], [3H], [14C] are used. One of large specific activity is stable preferred, and for example, as aforesaid enzyme, -galactosidase, -glucosidase, alkaline phosphatase, peroxidase, malate dehydrogenase are used. For example, as fluorescent material, fluorescamine, fluorescein thiocyanate are used. For example, as light generating substance, luminol, luminol derivative, luciferin, lucigenin are used. Furthermore a biotin-avidin system can be used to bind the antibody or an antigen with the labelling agent.

As far as the insolubilisation of the antigen or antibody is concerned, physical adsorption may be used, and moreover normally a process using chemical binding may be used, in order to insolubilise and immobilise the protein or enzyme. For example, as a carrier, agarose, dextran, insoluble polysaccharide such as cellulose, polystyrene, polyacrylamide, synthetic resin or for example silicon or glass is used.

In the sandwich method, the insolubilised monoclonal antibody of this invention is reacted with the test liquid (first reaction), and furthermore reacted with the labelled monoclonal antibody (second reaction) and thereafter, the ligand peptide of this invention or the receptor protein of this invention in the test liquid can be determined by measuring the activity of the labelling agent on the insolubilised carrier. The first reaction and the second reaction may be carried out in reverse order, or simultaneously carried out, or carried out at different times. The labeling agent and method of insolubilisation will now be described in greater detail.

In the immunity measuring method by sandwich method, antibody used with antibody for labelling or antibody for solid phase does not need to be always one kind, and a mixture of two kinds or more antibody may be used with the object of improving the sensitivity.

In measuring method of receptor proteins by sandwich method, for the monoclonal antibody of this invention used in the first reaction and the second reaction, antibodies with differing sites bound to the receptor protein are preferably used. In other words, as far as the antibody used in the first reaction and the second reaction are concerned, when the antibody used in the second

reactor recognises the C-terminal of the receptor protein, as the antibody used in the first reaction, an antibody which recognises other than C-terminal, preferably the N-terminal, is used.

Measurement system other than sandwich method, for example competition method, immunometric method or nephrometry can be used for the monoclonal antibody with respect to receptor proteins of this invention. In competition method, the labelled antigen and antigen in the test liquid are competitively reacted with antibody, and thereafter the labelled antigen (B) bonded with the antibody and the unreacted labelled antigen (F) are separated (B/F separation), and the labelled quantities of both B and F are measured, and the quantity of antigen in the test liquid is measured. In this reaction, a liquid phase method is used for the B/F separation, with insoluble antibody is used as antibody, and the use of for example polyethylene glycol and a second antibody with respect to the said antibody, or alternatively a solid phase immobilisation method is used, with the use of a solid immobilised antibody as the first antibody, or a soluble first antibody and an immobilised carrier as the second carrier.

In immunometric method, after carrying out a competitive reaction for a fixed quantity of labeling antibody with immobilised antigen and antigen in the test liquid, the solid phase and liquid phase are separated, or the antigen in the test liquid is reacted with excess labelled antibody, and thereafter the unreacted labelled antibody is bonded to the solid phase by the addition of solid phase immobilised antigen, and the solid phase and liquid phase separated. Thereafter a labelled quantity of either phase is measured, and the antigen quantity in test liquid is determined.

Moreover, in nephrometry, the quantity of insoluble sediment as a result of antigen-antibody reaction in gel or solution is measured. When there is a little quantity of antigen in the test liquid, and when only a little sediment is obtained, laser nephrometry utilising laser scattering is suitably used.

In the use of the various immunological measuring method as the measurement methods of this invention, the establishment of special conditions and procedures are not needed. As long as the ordinary technological considerations of a person skilled in the art applied in the ordinary way to each method, operation method, and the measurement system of receptor proteins of this invention or salts thereof is constructed. For details of the general technical procedures, the usual literature, books can be referred to [cf. for example, Hiroshi Irie ed. "radioimmunoassay"

(published by Kodansha, 1974), Hiroshi Irie ed. "radioimmunoassay, sequel" (published by Kodansha, 1975), Eiji Ishikawa et al. eds. "Enzyme assay" (Igakushoin, 1978), Eiji Ishikawa et al. eds. "Enzyme Immunoassay" (second edition) (Igakushoin, 1978), Eiji Ishikawa et al. eds. "Enzyme Immunoassay" (third edition) (Igakushoin, 1987), Eiji Ishikawa et al. eds. "Enzyme Immunoassay" (Igakushoin, 1978), Methods in Enzymology Vol. 70 Immunochemical Techniques, ditto Vol. 73 Immunochemical Techniques (Part B), ditto Vol. 74 Immunochemical Techniques (Part C), ditto Vol. 84 Immunochemical Techniques (Part D: Selected Immunoassays), ditto Vol. 92 Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods), ditto Vol. 121 Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies) (all published by Academic Press)].

As described above, by using antibody of this invention, it is possible to determine the receptor proteins of this invention or salts thereof with high accuracy.

Furthermore, using antibody of this invention, it is possible to diagnose various disease related to dysfunction of receptor protein of this invention by determining receptor proteins of this invention or salts thereof in-vivo.

Moreover antibody with respect to receptor proteins of this invention can be used in order to specifically detect receptor proteins of this invention present in analytes such as body fluid and tissue. Moreover the use is possible for the manufacture of antibody column used in order to purify receptor proteins of this invention, the detection of receptor protein of this invention in each fraction during purification, and the analysis of the behaviour of receptor proteins of this invention in test cells.

[10] Screening method of compound that changes the quantity of receptor protein of this invention or partial peptide thereof in cell membrane

Because the antibody of this invention can specifically recognise the of receptor protein of this invention or partial peptide thereof or salts thereof, it can be used for screening of compound that changes the quantity of receptor protein of this invention or partial peptide thereof in the membrane.

Namely, this invention puts forward for example,

(i) screening method for the compound that changes the quantity of receptor protein of this invention or partial peptide thereof on cell membrane by determining the quantity of receptor protein of this invention or partial peptide thereof contained in the cell membrane fraction obtained by isolating the cell membrane fraction after pulverising tissue or cells isolated from (1) blood, (2) specific organ (3) organs of non-human mammalian animals,

(ii) screening method for the compound that changes the quantity of receptor protein of this invention or partial peptide thereof on cell membrane by determining the quantity of receptor protein of this invention or partial peptide thereof contained in the cell membrane fraction obtained by isolating the cell membrane fraction after pulverising transformant that expressed of receptor protein of this invention or partial peptide thereof,

(iii) screening method for the compound that changes the quantity of receptor protein of this invention or partial peptide thereof on cell membrane, wherein pulverising tissue or cells isolated from (1) blood, (2) specific organ (3) organs of non-human mammalian animals is sectioned, and next by using immunostaining method, the degree of staining of said receptor protein on the cell surface layer is determined and said protein on the cell membrane is confirmed.

It also puts forward (iv) screening method for the compound that changes the quantity of receptor protein of this invention or partial peptide thereof on cell membrane, wherein pulverising transformant that expressed of receptor protein of this invention or partial peptide thereof is sectioned, and next by using immunostaining method, the degree of staining of said receptor protein on the cell surface layer is determined and said protein on the cell membrane is confirmed.

The determination of receptor protein of this invention or partial peptide thereof contained in cell membrane fraction is carried out as embodiment as follows.

(i) A drug (for example, anti-dementia drug, antihypertensive, anti-tumour agent, anti-obesity agent) or physical stress (for example water immersion stress, electric shock, light-dark, low temperature) is given to normal or disease model non-human mammalian animals (for example rat, mouse, rabbit, sheep, pig, bovine, cat, dog, monkey, more as embodiment, dementia rat, obese mouse, arteriosclerosis rabbit, tumour bearing mouse), a specified period has passed, and next, the tissue or cells isolated from blood or specific organ (for example brain, liver, kidney) or

organs is obtained. The obtained organ, tissue or cells are suspended in suitable buffer solution (for example, tris-hydrochloric acid buffer, phosphate buffer, hepes buffer), the organ, tissue or cells are pulverised, and by using surface active agent (for example Triton X100 [TM], Tween-80 [TM]) the cell membrane fraction is obtained by further using techniques such as centrifugation, filtration, column fractionation.

As the cell membrane fraction, cell is pulverised thereafter, a fraction containing much cell membrane which is obtained by itself familiar process is referred to. As method of pulverisation of cell, method to crush cells with Potter-Elvehjem type homogeniser, pulverisation with Waring blender and polytron (made by Kinematica Co.), pulverisation by supersonic wave, pulverisation by jetting cell from thin nozzle while pressurising it with French press are nominated. In fractionation of cell membrane, differential centrifugation method and fractionation by centrifugal force such as density-gradient centrifugation are mainly used. For example, cell breaking liquid is centrifuged at low speed (500 rpm - 3000 rpm) for a short time (for usually 1-10 minutes), and supernatant liquid is centrifuged for higher speed (15000 rpm - 30000 rpm) usually from 30 minutes to 2 hours, and the sedimentation which is obtained is regarded as the membrane fraction. In the said membrane fraction, receptor protein and phospholipid derived from cell and membrane constituent such as membrane protein are contained much.

The receptor protein of this invention or partial peptide thereof contained in cell membrane fraction can be determined by sandwich immunity measurement method using the antibody of this invention, Western blot analysis.

Such sandwich immunity measurement method can be carried out by the same aforesaid method, and Western blot can be carried out by itself well known method.

(ii) The transformant that expressed receptor protein of this invention or partial peptide thereof is produced in accordance with aforesaid method and the receptor protein of this invention or partial peptide thereof contained in the cell membrane fraction can be determined.

The screening of the compound that changes the quantity of receptor protein of this invention or partial peptide thereof can be carried out by method,

(i) wherein test compound is administered to the normal or disease model non-human mammalian animals at the same time or before giving drug or physical stress by a specified time (30 minutes before to 24 hours before, preferably 30 minutes before to 12 hours before, more preferably 1 hour before to 6 hours before) or after a specified period (30 minutes later to 3 days later, preferably 1 hour later to 2 days later, more preferably 1 hour later to 24 hours later), after a specified time has passed after administration (30 minutes later to 3 days later, preferably 1 hour later to 2 days later, more preferably 1 hour later to 24 hours later), the quantity of receptor protein of this invention or partial peptide thereof on cell membrane is determined,

(ii) wherein test compound is mixed in the culture medium during culturing of transformant by conventional method, after culturing for a specified time (1 day later to 7 days later, preferably 1 days later to 3 days later, more preferably 2 days later to 3 days later), the quantity of receptor protein of this invention or partial peptide thereof on cell membrane is determined.

The confirmation of receptor protein of this invention or partial peptide thereof contained in cell membrane fraction is carried out as follows.

(iii) A drug (for example, anti-dementia drug, antihypertensive, anti-tumour agent, anti-obesity agent) or physical stress (for example water immersion stress, electric shock, light-dark, low temperature) is given to normal or disease model non-human mammalian animals (for example rat, mouse, rabbit, sheep, pig, bovine, cat, dog, monkey, more as embodiment, dementia rat, obese mouse, arteriosclerosis rabbit, tumour bearing mouse), a specified period has passed, and next, the tissue or cells isolated from blood or specific organ (for example brain, liver, kidney) or organs is obtained. The obtained organ, tissue or cells are made into tissue section in accordance with usual method, and immunostaining is carried out by using the antibody of this invention. By determining the degree of staining of said receptor protein on the cell surface layer is determined and said protein on the cell membrane is confirmed, thereby the quantity of receptor protein of this invention or partial peptide thereof on cell membrane can be confirmed quantitatively or qualitatively.

(iv) Using transformant that expressed of receptor protein of this invention or partial peptide thereof, it can be confirmed by applying the same procedures.

The compound or salts thereof obtained by using screening method of this invention is a compound that has an action of changing the quantity of receptor protein of this invention or partial peptide thereof, and as embodiment, (a) compound that enhances the cell stimulation activity through G protein-coupled receptor protein (for example the activity to inhibit or the activity to promote arachidonic acid release, acetylcholine release, intracellular Ca²⁺ release, intracellular cGMP formation, inositol phosphate production, intracellular cAMP formation, cell membrane potential fluctuation, phosphorylation of intracellular protein, c-fos activation and lowering of pH) by increasing the quantity of receptor protein of this invention or partial peptide thereof on cell membranes and (b) compound that decreases said cell stimulation activity by decreasing the quantity of receptor protein of this invention or partial peptide thereof on cell membrane.

As said compound, peptide, protein, the non-peptide compound, synthetic compound, fermentation product are nominated and these compounds can be a novel compound or well known compound.

The compound that enhances said cell stimulation activity is useful as the medicine which has low toxicity for safely enhancing the physiological activity of receptor proteins of this invention.

The compound which decreases said cell stimulation activity is useful as the medicine which has low toxicity for safely decreasing the physiological activity of receptor proteins of this invention.

When the compound or the salts thereof which can be obtained by using screening process of this invention is used as aforesaid medicinal composition, it can be carried out according to usual practice. For example, in the same way as in aforesaid medicine containing receptor protein of this invention, it can be made into tablet, capsule agent, elixir medicine, microcapsule medicine, sterile solution, suspension.

Because drug obtained in this way is safe and low toxicity, can be administered for example to human and mammalian organisms (for example rat, rabbit, sheep, pig, bovine, cat, dog, monkey).

As for the dosage of the said compound or salts thereof, there is a difference depending on the administration subject, target organ, symptoms, administration method, but, in case of oral

administration, is from about 1.0-50 mg, preferably about 1.0-20 mg, preferably about 0.1-100 mg per day generally for example in schizophrenia patient (as 60 kg). When it is orally administered, dose per time thereof is different depending on administration subject, target organ, symptoms, administration method, but what is administered using an intravenous injection by about around 0.01-30 mg per day usually for example in schizophrenia patient (as 60 kg) in form of for example injection, 0.1-20 mg more preferably about around 0.1-10 mg is preferably convenient. In case of other animal, the quantity calculated as per 60 kg can be administered.

[11] Preventative and/or therapeutic agent of various illness which contains the compound which changes the quantity of receptor protein of this invention or partial peptide thereof

The receptor protein of this invention as mentioned above, is thought to play some important role in-vivo for example central function. Accordingly, the compound which changes the quantity of receptor protein of this invention or partial peptide thereof can be used as preventative and/or therapeutic agent of various illness related to dysfunction of receptor protein of this invention.

When said compound is used as preventative and/or therapeutic agent of various illness related to dysfunction of receptor protein of this invention, it can be carried out according to usual practice.

For example, the said compound can be used orally as the tablet applied with sugar coating in accordance with requirement, capsule agent, elixir medicine, microcapsule medicine or orally in the form of injection such as suspension or sterile solution of pharmacologically permitted liquid other than water. For example, it can be produced by admixture in the unit dosage form in a formulation with what is generally recognised as being a pharmacologically recognised well known carrier, flavouring agent, excipient, vehicle, antiseptic, stabiliser, binder. As for the quantity of effective ingredient in this formulation, a suitable quantity in a range as indicated by the field is obtained.

As the additive which can be admixed into a tablet, capsule agent, for example constituents such as gelatine, corn starch, tragacanth, gum Arabic, excipient such as crystalline cellulose, swelling agent such as corn starch, gelatine, alginic acid, lubricant such as magnesium stearate, flavouring agents such as a cherry, peppermint, akamono oil, sweetener such as saccharin or

sucrose, milk sugar were used. When dispensing unit form is capsule, in addition to material of aforesaid type, liquid state carrier such as oils and fats can be contained. A sterile composition for injection can be prescribed according to the ordinary formulation in which the active material and the naturally produced vegetable oil such as sesame oil, coconut in the vehicle such as water used for injection are suspended or dissolved. As aqueous liquid for injection, for example, isotonic liquid (for example D-sorbitol, D-mannitol, sodium chloride) including physiological saline, glucose and other supporting drug is used, and suitable solubiliser, for example alcohol (example, ethanol), polyalcohol (example, propylene glycol, polyethylene glycol), nonionic detergent (example, polysorbate 80 [TM], HCO-50) may be used together. As oily liquid, for example, sesame oil, soya bean oil are used, it may be used together with solubiliser such as benzyl benzoate, the benzyl alcohol.

Moreover aforesaid preventative and therapeutic agent can be formulated with for example buffer (for example phosphate buffer solution, sodium acetate buffer solution), analgesic (for example benzalkonium chloride, procaine hydrochloride), stabiliser (for example human serum albumin, polyethylene glycol), storage agent (for example benzyl alcohol, phenol), oxidation inhibitor. The injection which was prepared is packed into usually suitable ampoule.

Because drug obtained in this way is safe and low toxicity, can be administered for example to human and mammalian organisms (for example rat, rabbit, sheep, pig, bovine, cat, dog, monkey).

As for the dosage of for example ligand peptide of this invention, there is a difference depending on administration subject, target organ, symptoms, administration method, but in case of oral administration, for example, in schizophrenia patient (as 60 kg), it is about 0.1 mg-100 mg per day and is preferably about 1.0-50 mg and is more preferably about 1.0-20 mg generally. When it is orally administered, dose per time thereof is different depending on administration subject, target organ, symptoms, administration method, but it is about around 0.01-30 mg per day in usually for example in schizophrenia patient (as 60 kg) in form of injection and is preferably about around 0.1-20 mg, and is more preferably about around 0.1-10 mg and administration using an intravenous injection is convenient. In case of other animal, the quantity calculated as per 60 kg can be administered.

[12] Neutralisation by antibody with respect to receptor protein of this invention or partial peptide thereof or salts thereof

With the antibodies for the receptor protein of this invention or partial peptide thereof or salts thereof, by neutralisation activity for the their receptor proteins, there is meant namely the activity for the deactivation of the signal transduction function which said receptor protein participates in. Accordingly, when the said antibody has neutralising activity, the signal transduction related to the said receptor protein, for example the cell stimulating activity via the said receptor proteins (for example inhibiting or promoting activity, such as arachidonic acid release, acetylcholine release, Ca²⁺ release in cell, cGMP formation in cell, inositol phosphate production in cell, cAMP formation in cell, cell membrane potential fluctuation, phosphorylation of protein in cell, activation of c-fos, depression of pH) can be deactivated. Accordingly use is possible in the treatment and/or the prevention of disease originating in excess expression of said receptor protein.

[13] Production of non-human animals having DNA which codes G protein-coupled receptor protein of the invention

A transgenic non-human animal expressing receptor proteins of this invention can be produced using DNA of this invention. As an animal, mammalian organisms (for example rat, a mouse, a rabbit, a sheep, a pig, bovine, a cat, a dog, a monkey) (hereafter abbreviated as animal) can be proposed, and, a mouse, a rabbit are in particular suitable.

In the transfer of the DNA of this invention to target animals, the use of the said DNA as a gene construct which is bonded downstream of the promoter which can be expressed animal cells, is generally advantageous. For example, when transferring the DNA of this invention derived from rabbits, the gene construct wherein the DNA of this invention derived from an animal having high homology is bonded downstream of various promoters which can be expressed by the animal cells, can produce a DNA transfer animal which can mass produce the receptor proteins of this invention on the basis of microinjection into fertilised rabbit eggs. As this promoter, a ubiquitous expression promoter such as virus derived promoter for example metallothionein can be used, too, but NGF gene promoter or enolase gene promoter to specifically express in brain are preferably used.

In the fertilised egg cell level, the transfer of DNA of this invention can be guaranteed so that all of the somatic cell and object animal embryo cell are present. The presence of the receptor proteins in the embryonic cells of the produced animal after the DNA transfer, means that the offspring of the animal product have the receptor proteins of this invention in all the somatic cells and their embryonic cells. Animal descendants who inherited gene of this kind has receptor proteins of this invention in all of somatic cell and embryo cell thereof.

With an animal with the transferred DNA of this invention, it is confirmed that the genes are maintained with stability by mating, and breeding is passed in ordinary breeding environment as said DNA possession animal. Furthermore by mating male and female animals having the target gene, homozygotic animals having the introduced gene with both the homologous chromosomes may be obtained, and by mating these male and female animals, all the all the offspring will have the said DNA.

Animals having the transferred DNA of this invention have a high expression of the receptor proteins of this invention, and therefore are useful as for example animals for screening antagonist or agonists for receptor proteins of this invention.

DNA transfer animal of this invention can be used as the source of cell for tissue culture. For example, the DNA or RNA in the tissue of DNA transferred mice of this invention can be directly analysed, or by analysing tissue wherein receptor proteins of this invention have been expressed by genes are present, analysis of the receptor proteins of this invention can be carried out. Cells of tissue having the receptor proteins of this invention can be cultured by standard tissue culture techniques, and using these, for example it is possible to study the function of the cells from the generally difficult to culture tissue such as that derived from the brain or peripheral tissue. Moreover by using these cells, for example it is possible to select drugs such that the function of all tissue is increased. In addition, if there are high expression cell strains, receptor proteins of this invention can be are isolated and purified from these.

When base or amino-acid are indicated with abbreviation in chart and this specification, it is one on the basis of abbreviation or the usage abbreviation at aforesaid field, and the following make example thereof due to IUPAC-IUB Commission on Biochemical Nomenclature. Moreover when

there can be optical isomer on amino-acid, it makes L body is indicated if it is not in particular stated clearly.

DNA = deoxyribonucleic acid

cDNA = complementary deoxyribonucleic acid

A = adenine

T = thymine

G = guanine

C = cytosine RNA

RNA = ribonucleic acid

mRNA = messenger ribonucleic acid

dATP = deoxy adenosine triphosphate

dTTP = deoxy thymidine triphosphate

dGTP = deoxy guanosine triphosphate

dCTP = deoxy cytidine triphosphate

ATP = adenosine triphosphate

EDTA = ethylene diamine tetraacetic acid

SDS = sodium dodecyl sulphate

Gly = glycin

Ala = alanine

Val = valine

Leu = leucine

Ile = isoleucine

Ser = serine

Thr = threonine

Cys = cysteine

Met = methionine

Glu = glutamic acid

Asp = aspartic acid

Lys = lysine

Arg = arginine

His = histidine

Phe = phenylalanine

Tyr = tyrosine

Trp = triptophan

Pro = proline

Asn = asparagine

Gln = glutamine

pGlu = pyroglutamic acid

Me = methyl group

Et = ethyl group

Bu = butyl group

Ph = phenyl group

TC = thiazolidine -4 [R]- carboxamide thereof group

Moreover, the substitute groups, protecting groups and reagents frequently used in this specification are denoted by the following symbols.

Tos = p-toluene sulphonyl

CHO = formyl

Bzl = benzyl

Cl₂Bzl = 2,6- dichlorobenzyl

Bom = benzyl oxymethyl

Z = benzyl oxycarbonyl

Cl-Z = 2- chlorobenzyl oxycarbonyl

Br-Z = 2- bromobenzyl oxycarbonyl

Boc = t- butoxycarbonyl

DNP = dinitrophenol

Trt = trityl

Bom = t- butoxymethyl

Fmoc = N-9- fluorenylmethoxycarbonyl

HOBt = 1- hydroxy benz triazole

HOObt = 3,4- dihydro -3- hydroxy-4- oxo -1,2,3- benzotriazine

HONB = 1- hydroxy -5-norbornene -2,3-dicarboximide

DCC = N,N'- dicyclohexylcarbodiimide

Sequence number of sequence list of this specification denotes the following sequences.

[sequence number: 1]

Amino acid sequence of rat cerebellum derived novel G protein-coupled receptor protein rCB7T084 of this invention is shown.

[sequence number: 2]

Base sequence of cDNA coding for rat cerebellum derived novel G protein-coupled receptor protein rCB7T084 of this invention having amino acid sequence represented by sequence number: 1 is shown.

[sequence number: 3]

Base sequence of primer 1 which was used for cloning cDNA coding for rat cerebellum derived novel G protein-coupled receptor protein rCB7T084 is shown.

[sequence number: 4]

Base sequence of primer 2 which was used for cloning cDNA coding for rat cerebellum derived novel G protein-coupled receptor protein rCB7T084 is shown.

As for transformant *Escherichia coli* DH10B/pAK-rCB084 obtained in Example 1 mentioned later, it is deposited as deposition number FERM BP-6485 at Ministry of trade and industry, Industrial Technology Bureau, Bioengineering Industrial Technology Laboratory (NIBH) from September 4, 1998, and is deposited as deposition number IFO 16199 at Fermentation Laboratory Foundation (IFO) from August 17, 1998.

Below Example and reference Example are shown, and this invention is described in greater detail, but this is not one limiting a range of this invention. Moreover gene operation using *E. coli* were carried out in accordance with molecular cloning.

Example 1

Cloning of cDNA coding for rat cerebellum derived G protein-coupled receptor protein and determination of base sequence

Rat cerebellum cDNA was used as a template, and PCR reaction was carried out using 2 primers, namely primer 1 (sequence number: 3) and primer 2 (sequence number: 4). Composition of the reaction liquor in said reaction used a one-tenth quantity of aforesaid cDNA as a template, and 0.2 μ M each of primer 1 (sequence number: 4) and primer 2 (sequence number: 5), dNTPs 200 μ M, and Advantage cDNA Polymerase Mix (CLONTECH Co.) 1/50 quantity were added to buffer attached to enzyme, and it was made up to the liquid quantity of 50 μ l. As PCR reaction, (1) 95 degrees•30 seconds, thereafter (2) cycle of 94 degrees•5 seconds, 70 degrees•5 minutes repeated 5 times, (3) cycle of 94 degrees•5 seconds, 68 degrees•5 minute repeated 5 times, (4) cycle of 94 degrees•5 seconds, 65 degrees•5 minutes was repeated 35 times, and finally (5) elongation reaction of 65 degrees•5 minutes was carried out. Said PCR reaction was made, and next reaction product was subcloned to plasmid vector pCR II (Invitrogen Co.) according to formulation of TA cloning kit (Invitrogen Co.). This was introduced to *E. coli* DH5 , and the clone having cDNA was selected in LB agar medium containing ampicillin and next having analysed sequence of clone, as a result, cDNA sequence which coded for novel G protein-coupled receptor protein (sequence number: 2) was obtained. The novel G protein-coupled receptor protein containing amino acid sequence derived from this cDNA (sequence number: 1) was named rCB7T084.

The plasmid pAK-rCB084 in which cDNA coding for the rat cerebellum derived novel G protein-coupled receptor protein rCB7T084 (sequence number: 2) has been subcloned, was introduced into *Escherichia coli* DH10B in accordance with well known method, and a transformant: *Escherichia coli* DH10B/pAK-rCB084 was obtained.

Moreover, the plasmid pCR II-rCB7T084 in which cDNA coding for the rat cerebellum derived novel G protein-coupled receptor protein rCB7T084 (sequence number: 2) has been subcloned, was introduced into *Escherichia coli* DH5 in accordance with well known method, and a transformant: *Escherichia coli* DH5 /pCR II-rCB7T084 was obtained.

Example 2

Production of rCB7T084-expression CHO cells

The transformant *E. coli* DH5 /pCR II-rCB7T084 produced in Example 1, was cultured, and next plasmid DNA of pCR II-rCB7T084 was prepared using plasmid midkit (Qiagen Co.). The cDNA

coding for G protein-coupled receptor protein rCB7T084 of this invention was cloned from this plasmid into plasmid vector pcDNA3.1/V5.His for protein expression, and plasmid pcDNA3.1-rCB084 for protein expression was constructed. A large quantity of plasmid DNA was prepared using plasmid midkit (Qiagen Co.), thereafter plasmid obtained in this way was introduced into CHO dhfr- cells in accordance with the attached protocol using CellPfect Transfection Kit (Amersham Pharmacia Biotech Co.). In other words, the DNA 10 mg was made into a co-precipitated suspension with calcium phosphate, and added to 10 cm petri dish to which CHO dhfr- cells 5×10^5 or 1×10^6 cells were inoculated 24 hours earlier, and next, the culturing was carried out on MEM medium containing 10 % bovine foetus serum for 1 day, thereafter subculturing was carried out, and next the culturing was carried out on a selective medium, MEM medium containing 10 % bovine foetus serum and 1 mg/ml G418 (Gibco BRL Co.). The colonies of transformed cell (CHO/rCB084) that proliferated on the selective medium were selected, thereby rCB7T084-expression CHO cells were obtained.

The total RNA was extracted from the selected rCB7T084-expression CHO cells in accordance with conventional method, thereafter, mRNA quantity of rCB7T084 was measured by TaqMan method, and the number of copies was calculated. The results are shown in the Table below.

Clone No.	Expression quantity (copies /ng total RNA)	
	1st measurement	2nd measurement
3	18666	17496
	18278	24369
4	181736	190185
	159347	175345
17	50429	34503
	44990	41239

Possible applications in industry

G protein-coupled receptor protein of this invention or partial peptides thereof or salts thereof and the polynucleotide coding for these (for example DNA, RNA and those derivatives) can be used for, (1) determination of ligand (agonist), (2) acquisition of antiserum and antibody, (3) assembly of expression system of recombination type receptor protein, (4) screening of drug candidate compound and development of the receptor binding assay system which used said expression system, (5) operation of drug design on the basis of reference of structurally similar ligand receptor, (6) agent for making PCR primer and probe at genetic screening, (7) transgenic animal manufacture, or (8) medicine of for example gene preventative and therapeutic agent.

Limits of the Claims

1. A G protein-coupled receptor protein or the salts thereof which is characterised by containing amino acid which is the same or substantially same amino acid sequence as represented by sequence number: 1.
2. Partial peptide or salts thereof of the G protein-coupled receptor protein in accordance with Claim 1.
3. The polynucleotide which contains polynucleotide having base sequence coding for the G protein-coupled receptor protein in accordance with Claim 1.
4. Polynucleotide in accordance with the Claim 3 which is DNA.

5. Polynucleotide in accordance with Claim 3 having base sequence represented by sequence number: 2.
6. Recombinant vector containing the polynucleotide in accordance with Claim 3
7. The transformant which was transformed with recombinant vector in accordance with Claim 6.
8. A process for the production of the G protein-coupled receptor protein in accordance with Claim 1 or salts thereof to be characterised in that transformant in accordance with Claim 7 is cultured, and the G protein-coupled receptor protein in accordance with Claim 1 is formed.
9. Antibody with respect to G-G protein-coupled receptor protein coupled receptor G protein-coupled receptor protein in accordance with Claim 1, partial peptide in accordance with Claim 2 or salts thereof.
10. Antibody in accordance with Claim 9 that is a neutralising antibody that inactivates signal transduction of the G protein-coupled receptor protein in accordance with Claim 1.
11. The diagnosis drug which is obtained by containing antibody in accordance with Claim 9.
12. Ligand with respect to the G protein-coupled receptor protein or salts thereof in accordance with Claim 1 that can be obtained by using the G protein-coupled receptor protein in accordance with Claim 1, or using partial peptide in accordance with Claim 2 or salts thereof.
13. The medicine containing the ligand in accordance with Claim 12.
14. Determination method of ligand with respect to the G protein-coupled receptor protein or salts thereof in accordance with Claim 1 to be characterised by using the G protein-coupled receptor protein in accordance with Claim 1 or partial peptide in accordance with Claim 2 or salts thereof.
15. Screening process of the compound or salts thereof that changes binding of ligand and the G protein-coupled receptor protein in accordance with Claim 1 or salts thereof to be characterised

by using the G protein-coupled receptor protein in accordance with Claim 1, or partial in accordance with Claim 2 or salts thereof.

16. A kit for screening of the compound or salts thereof that changes binding of ligand and the G protein-coupled receptor protein in accordance with Claim 1 or salts thereof to be characterised by containing the G protein-coupled receptor protein in accordance with Claim 1, or partial peptide in accordance with Claim 2 or salts thereof.

17. The compound or salts thereof which can change the binding of the ligand and the G protein-coupled receptor protein in accordance with Claim 1 or salts thereof, which can be obtained using the screening process in accordance with Claim 15 or the kit for screening in accordance with Claim 16.

18. The medicine containing the compound or salts thereof to change ligand and binding of the G protein-coupled receptor protein in accordance with Claim 1 or salts thereof, obtained using the screening process in accordance with Claim 15 or the kit for screening in accordance with Claim 16.

19. Polynucleotide that hybridises with polynucleotide in accordance with Claim 3 under highly stringent condition.

20. Polynucleotide that contains base sequence complementary to polynucleotide in accordance with Claim 3 or a part thereof.

21. Determination method of mRNA of the G protein-coupled receptor protein in accordance with Claim 1 to be characterised by using to polynucleotide in accordance with Claim 3 or a part thereof.

22. Determination method of the G protein-coupled receptor protein in accordance with Claim 1 to be characterised by using the antibody in accordance with Claim 9.

23. Diagnosis method of diseases related to the function of the G protein-coupled receptor protein in accordance with Claim 1 to be characterised by using the determination method in accordance with Claim 21 or Claim 22.

24. Screening process of compound that changes the quantity of expression of the G protein-coupled receptor protein in accordance with Claim 1 to be characterised by using the determination method in accordance with Claim 21.

25. Screening process of compound that changes the quantity of the G protein-coupled receptor protein in accordance with Claim 1 on cell membrane to be characterised by using the determination method in accordance with Claim 22.

26. Compound that changes the quantity of expression of the G protein-coupled receptor protein in accordance with Claim 1 or salts thereof that can be obtained by screening process in accordance with Claim 24.

27. Compound that changes the quantity of the G protein-coupled receptor protein in accordance with Claim 1 on cell membrane that can be obtained by screening process in accordance with Claim 25, or salts thereof.

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