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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 15/12, C07K 13/00
C12N 5/10, G01N 33/74

A1

(11) International Publication Number: **WO 93/19175**

(43) International Publication Date: 30 September 1993 (30.09.93)

(21) International Application Number: PCT/EP93/00697

(22) International Filing Date: 23 March 1993 (23.03.93)

(30) Priority data:
398/92 25 March 1992 (25.03.92) DK

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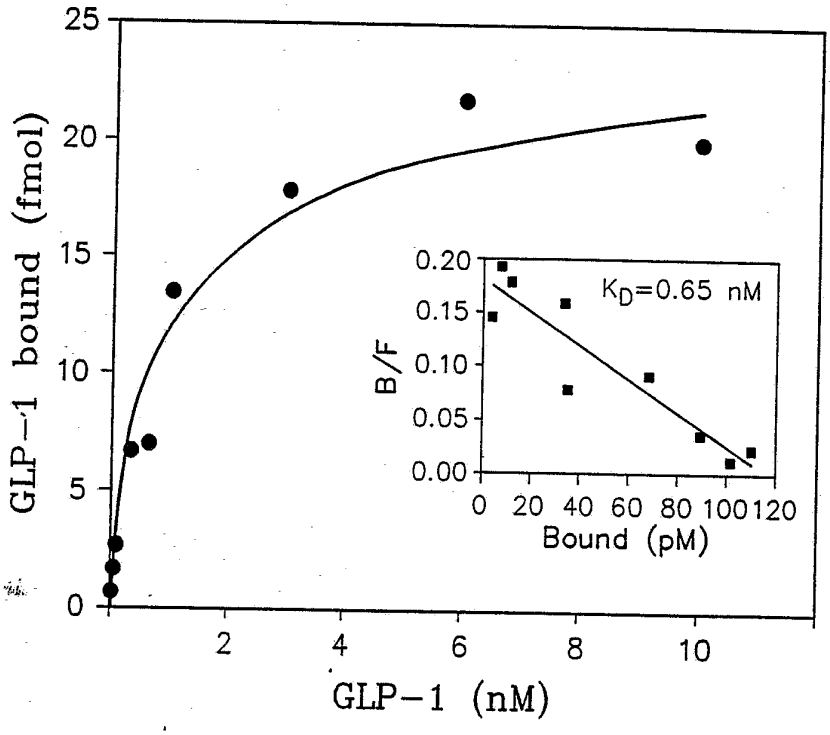
(81) Designated States: AU, BB, BG, BR, CA, CZ, FI, HU, JP, KP, KR, KZ, LK, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: RECEPTOR FOR THE GLUCAGON-LIKE-PEPTIDE-1 (GLP-1)



(57) Abstract

The present invention relates to a recombinant glucagon-like peptide-1 (GLP-1) receptor, to a DNA construct which comprises a DNA sequence encoding a GLP-1 receptor, to methods of screening for agonists of GLP-1 activity, and to the use of the GLP-1 receptor for screening for agonists of GLP-1 activity.

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RECEPTOR FOR THE GLUCAGON-LIKE-PEPTIDE-1 (GLP-1)

FIELD OF THE INVENTION

The present invention relates to a recombinant glucagon-like peptide-1 (GLP-1) receptor, to a DNA construct which comprises a DNA sequence encoding a GLP-1 receptor, to methods of screening for agonists of GLP-1 activity, and to the use of the GLP-1 receptor for screening for agonists of GLP-1 activity.

BACKGROUND OF THE INVENTION

As used in the present specification the designation GLP-1 comprises GLP-1(7-37) as well as GLP-1(7-36)amide.

Glucose-induced insulin secretion is modulated by a number of hormones and neurotransmitters. In particular, two gut hormones, glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) potentiate the effect of glucose on insulin secretion and are thus called gluco-incretins (Dupre, in The Endocrine Pancreas, E. Samois Ed. (Raven Press, New York, (1991), 253 - 281) and Ebert and Creutzfeld, (Diabetes Metab. Rev. 3, (1987)). Glucagon-like peptide-1 is a gluco-incretin both in rat and in man (Dupre and Ebert and Creutzfeld, vide supra, and Kreymann et al. (Lancet 2 (1987), 1300)). It is part of the preproglucagon molecule (Bell et al. Nature 304 (1983), 368) which is proteolytically processed in intestinal L cells to GLP-1(1-37) and GLP-1(7-36)amide or GLP-1(7-37) (Mojsov et al. (J.Biol.Chem. 261 (1986), 11880) and Habener et al.: The Endocrine Pancreas E. Samois Ed. (Raven Press, New York (1991), 53 - 71). Only the truncated forms of GLP-1 are biologically active and both have identical effects on insulin secretion in beta cells (Mojsov et al. J.Clin.Invest 79 (1987), 616) and Weir et al. (Diabetes 38 (1989), 338). They are the most potent gluco-incretins so far described and are

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active at concentrations as low as one to ten picomolar. The stimulatory effect of these gluco-incretin hormones requires the presence of glucose at or above the normal physiological concentration of about 5 mM and is mediated by activation of adenylate cyclase and a rise in the intracellular concentration of cyclic AMP (Drucker et al. Proc.Natl.Acad.Sci. USA 84 (1987), 3434) and Göke et al. (Am.J.Physiol. 257 (1989), G397). GLP-1 has also a stimulatory effect on insulin gene transcription (Drucker et al. Proc.Natl.Acad.Sci. USA 84 (1987), 3434). In a rat model of non-insulin-dependent diabetes mellitus (NIDDM) is associated with a reduced stimulatory effect of GLP-1 on glucose-induced insulin secretion (Suzuki et al. Diabetes 39 (1990), 1320). In man, in one study, GLP-1 levels were elevated in NIDDM patients both in the basal state and after glucose ingestion; however, following a glucose load there was only a very small rise in plasma insulin concentration (Ørskov et al. J.Clin.Invest. 87 (1991), 415). A recent study (Nathan et al. Diabetes Care 15 (1992), 270) showed that GLP-1 infusion could ameliorate postprandial insulin secretion and glucose disposal in NIDDM patients. Thus, as a further step in understanding the complex modulation of insulin secretion by gut hormones and its dysfunction in diabetes, we isolated and characterized a complementary DNA for the beta cell GLP-1 receptor and showed that it is part of a new family of G-coupled receptors.

DESCRIPTION OF THE INVENTION

The present invention relates to a recombinant glucagon-like peptide-1 (GLP-1) receptor.

More preferably, the invention relates to a GLP-1 receptor which comprises the amino acid sequence shown in SEQ ID No. 1, or an analogue thereof binding GLP-1 with an affinity constant, K_D, below 100 nM, preferably below 10 nM. In the present

context, the term "analogue" is intended to indicate a naturally occurring variant (including one expressed in other animal species, in particular human) of the receptor or a "derivative" i.e. a polypeptide which is derived from the native GLP-1 receptor by suitably modifying the DNA sequence coding for the variant, resulting in the addition of one or more amino acids at either or both the C- and N-terminal ends of the native amino acid sequence, substitution of one or more amino acids at one or more sites in the native amino acid sequence, deletion of one or more amino acids at either or both ends of the native sequence or at one or more sites within the native sequence, or insertion of one or more amino acids in the native sequence.

In another aspect, the present invention relates to a DNA construct which comprises a DNA sequence encoding the GLP-1 receptor of the invention, as well as a recombinant expression vector carrying the DNA construct and a cell containing said recombinant expression vector.

In one embodiment of the invention, the GLP-1 receptor molecule may be provided in solubilised and/or reconstituted form.

In the present context "solubilised" is intended to indicate a receptor as present in detergent-solubilised membrane preparations. "Reconstituted" is intended to indicate a receptor solubilised in the prescence of essential cofactors, e.g. G-protein. In this embodiment the receptor may be in a reconstituted micellar form.

The DNA construct of the invention encoding the GLP-1 receptor preferably comprises the DNA sequence shown in SEQ ID No. 1, or at least a DNA sequence coding for a functional analogue thereof binding GLP-1 with an affinity below 100 nM, preferably below 10 nM or a suitable modification thereof. Examples of suitable modifications of the DNA sequence are nucleotide

substitutions which do not give rise to another amino acid sequence of the GLP-1 receptor, but which may correspond to the codon usage of the host organism into which the DNA construct is introduced or nucleotide substitutions which do give rise to a different amino acid sequence and therefore, possibly, a different protein structure without, however, impairing the properties of the native variant. Other examples of possible modifications are insertion of one or several nucleotides into the sequence, addition of one or several nucleotides at either end of the sequence, or deletion of one or several nucleotides at either end or within the sequence.

Another example of a DNA construct of the invention is one which encodes a GLP-1 receptor variant particularly suitable for solubilisation and reconstitution.

The DNA construct of the invention encoding the present GLP-1 receptor may be prepared synthetically by established standard methods, e.g. the phosphoamidite method described by Beaucage and Caruthers, Tetrahedron Letters 22 (1981), 1859 - 1869, or the method described by Matthes et al., EMBO Journal 3 (1984), 801 - 805. According to the phosphoamidite method, oligonucleotides are synthesized, e.g. in an automatic DNA synthesizer, purified, annealed, ligated and cloned in suitable vectors.

The DNA construct of the invention may also be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the GLP-1 receptor of the invention by hybridization using synthetic oligonucleotide probes in accordance with standard techniques (cf. Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989). In this case, a genomic or cDNA sequence encoding the GLP-1 receptor may be modified at a site corresponding to the site(s) at which it is desired to introduce amino acid substitutions, e.g. by

site-directed mutagenesis using synthetic oligonucleotides encoding the desired amino acid sequence for homologous recombination in accordance with well-known procedures.

Finally, the DNA construct may be of mixed synthetic and genomic, mixed synthetic and cDNA or mixed genomic and cDNA origin prepared by ligating fragments of synthetic, genomic or cDNA origin (as appropriate), the fragments corresponding to various parts of the entire DNA construct, in accordance with standard techniques. The DNA construct may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or Saiki et al., Science 239 (1988), 487 - 491.

The recombinant expression vector into which the DNA construct of the invention is inserted may be any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the DNA sequence encoding the GLP-1 receptor of the invention should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA encoding the GLP-1 receptor of the invention in mammalian cells are the SV40 promoter (Subramani et al., Mol. Cell Biol. 1 (1981), 854 -

864), the MT-1 (metallothionein gene) promoter (Palmiter et al., Science 222 (1983), 809 - 814) or the adenovirus 2 major late promoter. A suitable promoter for use in insect cells is the polyhedrin promoter (Vasuvedan et al., FEBS Lett. 311, (1992) 7 - 11). Suitable promoters for use in yeast host cells include promoters from yeast glycolytic genes (Hitzeman et al., J. Biol. Chem. 255 (1980), 12073 - 12080; Alber and Kawasaki, J. Mol. Appl. Gen. 1 (1982), 419 - 434) or alcohol dehydrogenase genes (Young et al., in Genetic Engineering of Microorganisms for Chemicals (Hollaender et al, eds.), Plenum Press, New York, 1982), or the TPI1 (US 4,599,311) or ADH2-4c (Russell et al., Nature 304 (1983), 652 - 654) promoters. Suitable promoters for use in filamentous fungus host cells are, for instance, the ADH3 promoter (McKnight et al., The EMBO J. 4 (1985), 2093 - 2099) or the tpiA promoter.

The DNA sequence encoding the GLP-1 receptor of the invention may also be operably connected to a suitable terminator, such as the human growth hormone terminator (Palmiter et al., op. cit.) or (for fungal hosts) the TPI1 (Alber and Kawasaki, op. cit.) or ADH3 (McKnight et al., op. cit.) terminators. The vector may further comprise elements such as polyadenylation signals (e.g. from SV40 or the adenovirus 5 Elb region), transcriptional enhancer sequences (e.g. the SV40 enhancer) and translational enhancer sequences (e.g. the ones encoding adenovirus VA RNAs).

The recombinant expression vector of the invention may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. An example of such a sequence (when the host cell is a mammalian cell) is the SV40 origin of replication. The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the gene coding for dihydrofolate reductase (DHFR) or one which confers resistance to a drug, e.g. neomycin, hygromycin or methotrexate.

The procedures used to ligate the DNA sequences coding for the GLP-1 receptor of the invention, the promoter and the terminator, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance, Sambrook et al., op.cit.).

The host cell into which the expression vector of the invention is introduced may be any cell which is capable of producing the GLP-1 receptor of the invention and is preferably a eukaryotic cell, such as invertebrate (insect) cells or vertebrate cells, e.g. Xenopus laevis oocytes or mammalian cells, in particular insect and mammalian cells. Examples of suitable mammalian cell lines are the COS (ATCC CRL 1650), BHK (ATCC CRL 1632, ATCC CCL 10), CHL (ATCC CCL39) or CHO (ATCC CCL 61) cell lines. Methods of transfecting mammalian cells and expressing DNA sequences introduced in the cells are described in e.g. Kaufman and Sharp, J. Mol. Biol. 159 (1982), 601 - 621; Southern and Berg, J. Mol. Appl. Genet. 1 (1982), 327 - 341; Loyter et al., Proc. Natl. Acad. Sci. USA 79 (1982), 422 - 426; Wigler et al., Cell 14 (1978), 725; Corsaro and Pearson, Somatic Cell Genetics 7 (1981), 603, Graham and van der Eb, Virology 52 (1973), 456; and Neumann et al., EMBO J. 1 (1982), 841 - 845.

Alternatively, fungal cells (including yeast cells) may be used as host cells of the invention. Examples of suitable yeasts cells include cells of Saccharomyces spp. or Schizosaccharomyces spp., in particular strains of Saccharomyces cerevisiae. Examples of other fungal cells are cells of filamentous fungi, e.g. Aspergillus spp. or Neurospora spp., in particular strains of Aspergillus oryzae or Aspergillus niger. The use of Aspergillus spp. for the expression of proteins is described in, e.g., EP 272 277.

The GLP-1 receptor according to the invention may be produced by a method which comprises culturing a cell as described above

in a suitable nutrient medium under conditions which are conducive to the expression of the GLP-1 receptor, and recovering the GLP-1 receptor from the culture. The medium used to culture the cells may be any conventional medium suitable for growing mammalian cells, such as a serum-containing or serum-free medium containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection).

If the GLP-1 receptor has retained the transmembrane and (possibly) the cytoplasmic region of the native variant, it will be anchored in the membrane of the host cell, and the cells carrying the GLP-1 receptor may be used as such in the screening or diagnostic assay. Alternatively, the receptor may be a component of membrane preparations, e.g. in solubilised and/or reconstituted form as defined above.

In a still further aspect, the present invention relates to a method of screening for agonists or enhancers of GLP-1 activity, the method comprising incubating a GLP-1 receptor according to any of claims 1 - 3 with a substance suspected to be an agonist of GLP-1 activity and subsequently with a GLP-1 or an analogue thereof, and detecting any effect from the suspected agonist on the binding of GLP-1 to the GLP-1 receptor. An enhancer being defined as a compound capable of stabilizing interaction between a high-affinity form of the receptor and the corresponding ligand, as described e.g. for the adenosin receptor (Bruns et al. Molecular Pharmacology 38 (1990), 939).

An alternative method of screening for agonists of GLP-1 activity, comprises incubating GLP-1 or an analogue thereof with a substance suspected to be an agonist of GLP-1 activity and subsequently with a GLP-1 receptor of the invention, and detecting any effect on the binding to the GLP-1 receptor. Such

agonists of GLP-1 activity will be substances stimulating glucose-induced insulin secretion and may be used in the treatment of NIDDM.

The GLP-1 receptor may be immobilized on a solid support and may, as such, be used as a reagent in the screening methods of the invention. The GLP-1 receptor may be used in membrane-bound form, i.e. bound to whole cells or as a component of membrane preparations immobilised on a solid support.

The solid support employed in the screening methods of the invention preferably comprises a polymer. The support may in itself be composed of the polymer or may be composed of a matrix coated with the polymer. The matrix may be of any suitable material such as glass, paper or plastic. The polymer may be selected from the group consisting of a plastic (e.g. latex, a polystyrene, polyvinylchloride, polyurethane, polyacrylamide, polyvinylalcohol, nylon, polyvinylacetate, and any suitable copolymer thereof), cellulose (e.g. various types of paper, such as nitrocellulose paper and the like), a silicon polymer (e.g. siloxane), a polysaccharide (e.g. agarose or dextran), an ion exchange resin (e.g. conventional anion or cation exchange resins), a polypeptide such as polylysine, or a ceramic material such as glass (e.g. controlled pore glass).

The physical shape of the solid support is not critical, although some shapes may be more convenient than others for the present purpose. Thus, the solid support may be in the shape of a plate, e.g. a thin layer or microtiter plate, or a film, strip, membrane (e.g. a nylon membrane or a cellulose filter) or solid particles (e.g. latex beads or dextran or agarose beads). In a preferred embodiment, the solid support is in the form of wheat germ agglutinin-coated SPA beads (cf. US 4,568,649).

Alternatively, screening for GLP-1 agonists can also be carried out using a cell line expressing the cloned GLP-1 receptor functionally coupled to a G-protein. In living cells, exposure to an agonist will give rise to an increase in the intracellular cAMP concentration. The cAMP concentration can then be measured directly. Changes in cAMP levels may also be monitored indirectly using appropriate cell lines in which a measurable signal is generated in response to an increase in intracellular cAMP.

It is furthermore contemplated to locate the ligand-binding site on the GLP-1 receptor of the invention, for instance by preparing deletion or substitution derivatives of the native GLP-1 receptor (as described above) and incubating these with ligands known to bind the full-length GLP-1 receptor and detecting any binding of the ligand to the GLP-1 receptor deletion derivative. Once the ligand-binding site has been located, this may be used to aquire further information about the three-dimensional structure of the ligand-binding site. Such three-dimensional structures may, for instance, be established by means of protein engineering, computer modelling, NMR technology and/or crystallographic techniques. Based on the three-dimensional structure of the ligand-binding site, it may be possible to design substances which are agonists to the GLP-1 molecule.

The characterization of the GLP-1 receptor is of considerable physiological and pathological importance. It will help study a fundamental aspect of the entero-insular axis (Unger and Eisentraut, Arch.Int.Med. 123 (1969), 261): the potentiating effect of gut hormones on glucose-induced insulin secretion, the role of these hormones in the control of glucose homeostasis and also the possible therapeutic use of GLP-1 to stimulate insulin secretion in NIDDM patients (Mathan et al. Diabetes Care 15 (1992), 270). Investigation of the regulated expression and desensitization of the receptor in the normal

state and during the development of diabetes will contribute to a better understanding of the modulation of insulin secretion in normal and pathological situations. Availability of antibodies against this receptor may also allow an analysis of the surface localization of this receptor and its distribution relative to the beta cell glucose transporter GLUT2 (Thorens et al. Cell 55 (1988), 281 and Orci et al. Science 245 (1989), 295). This aspect pertains to the hypothesis that the beta cell membrane has a "regulatory" domain which contains hormone receptors (Bonner-Weir Diabetes 37 (1988), 616), and which may be distinct from GLUT2-containing membrane domains previously identified (Thorens et al. Cell 55 (1988), 281 and Orci et al. Science 245 (1989), 295). Finally, the identification of an additional member of this new family of G-coupled receptors will help design experiments to probe the structure-function relationship of these new molecules.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is further illustrated in the following examples with reference to the appended drawings in which

Fig. 1A and Fig. 1B which is a continuation of Fig. 1A together show the amino acid sequence of the rat GLP-1 receptor in a comparison with the sequence of the rat secretin receptor (SECR), the opossium parathyroid hormone receptor (PTHR) and the porcine calcitonin receptor (CTR1). The GLP-1 receptor has three N glycosylation sites in the extracellular domain (arrows). Four cysteines are conserved at identical places in the four receptor (boxes). Note the otherwise very divergent sequences in this part of the molecules as well as in the COOH-terminal cytoplasmic tail. Sequence identities are denoted by stars and homologies by dots. The location of the putative transmembrane domains are indicated by horizontal bars above the sequences.

Fig. 2 shows binding of ^{125}I-GLP-1 to COS cells transfected with the pGLPR-16 plasmid. Specific binding reaches saturation at 1 to 10 nM GLP-1. Insert: Scatchard analysis of GLP-1 binding.

Fig. 3 shows binding of ^{125}I-GLP-1 to INS-1 cells. Specific binding reaches saturation at 1 to 10 nM GLP-1. Insert: Scatchard analysis of GLP-1 binding.

Fitting of the curves in Figs. 2 and 3 were performed with the LIGAND program (McPherson, Kinetic, EBDA, Ligand, Lowry. A Collection of radioligand analysis programs (Elsevier, Amsterdam, 1985)).

Fig. 4 shows displacement of ^{125}I-GLP-1 binding to COS cells transfected with the rat GLP-1 receptor cDNA. Transfected cells were incubated with 20 pM ^{125}I-GLP-1 in the presence of increasing concentrations of cold peptides. Each point was measured in duplicate and the experiments repeated three times for GLP-1, GIP and glucagon and once for VIP and secretin.

Fig. 5 shows stimulation of cyclic AMP formation in COS cells transfected with the rat GLP-1 receptor cDNA. COS cells were transfected with the pcDNA-1 vector alone (open bars) or the pGLPR-1 plasmid (stripped bar) and incubated in the absence or the presence of GLP-1 at the indicated concentration. cAMP production was measured in triplicate with a radioimmunoassay (Amersham).

Fig. 6 shows tissue specificity of GLP-1 receptor expression assessed by Northern blotting of RNA from different tissues and from the INS-1 cell line. Ten micrograms of total RNA was analyzed on each lane. Two major RNA species of 2.7 and 3.6 kb were detected in all tissues in which the receptor was detected. The position of the migration of the ribosomal RNAs is indicated to the left of the picture.

Fig. 7 is a comparison of the rat GLP-1 receptor amino acid sequence (rat) and a partial amino acid sequence of the human GLP-1 receptor (human).

The present invention is further illustrated in the following examples which is not intended to be in any way limiting to the scope of the invention as claimed.

EXAMPLE 1

Molecular Cloning and Characterisation of the Rat Islet GLP-1 Receptor cDNA.

A rat pancreatic islet cDNA library was constructed in the pcDNA-1 expression vector (Rat pancreatic islets were prepared according to Gotoh et al. (Transplantation 43 (1985), 725). PolyA+ RNA was prepared and the cDNA library was constructed in the pcDNA-1 vector (In Vitrogen) as described by Aruffo and Seed (Proc.Natl.Acad.Sci. USA 84 (1987), 8573) and Lin et al. (Proc.Natl.Acad.Sci. USA 88 (1991), 3185). Plasmid DNA was prepared from pools of five to eight thousands bacterial clones (Maniatis et al., Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, 1982) and transfected into COS cells (Sompayrac and Dana, Proc.Natl.Acad.Sci. USA 78 (1981), 7575). The presence of GLP-1 receptor expressed in COS cells was assessed by binding of the radioiodinated peptide followed by photographic emulsion autoradiography and screening by dark field microscopy (Gearing et al. EMBO J. 8 (1989), 3667). GLP-1(7-36)amide, as well as the other peptides, were purchased from Peninsula Laboratories. Iodination was performed by the iodine monochloride method (Contreras et al. Meth.Enzymol. 92 (1983), 277), the peptide was purified by passage over Sephadex G-10 followed by CM-Sepharose and specific activity was determined by the self displacement technique (Calvo et al. Biochem. 212 (1983), 259). A 1.6 kb cDNA clone (pGLPR-1) was isolated by subfractionation of an original positive pool and

was used to isolate, by DNA hybridization screening, two additional clones from primary positive pools. These plasmids (pGLPR-16 and -87) had inserts of 3.0 and 2.0 kb, respectively. Transfection of these clones into COS cells generated high affinity (K_D = 0.6 nM) binding sites for GLP-1 (Fig. 2). This affinity is comparable to that seen for binding of GLP-1 to the rat insulinoma cell line INS-1 (Asfari et al. Endocrinology 130 (1992), 167) (K_D = 0.12 nM; Fig. 3). In both cases a single high affinity binding component was detected. The binding to GLP-1 receptor transfected COS cells reached a plateau between 1 and 10 nM. At concentrations above 10 nM a second, high capacity, low affinity, binding component was detected. Although specifically displacable by cold GLP-1, this binding was also present in COS cells transfected with the expression vector alone and was therefore not further characterized.

Binding of GLP-1 to the receptor expressed in COS cells was displaced by cold GLP-1 with a 50 percent displacement achieved at 0.5 to 1 nM (Fig. 4). Other peptide hormones of related structure such as secretin, gastric inhibitory peptide (GIP) and vasoactive intestinal peptide (VIP) (Dupre in The Endocrine Pancreas, E. Samois Ed. (Raven Press, New York, (1991), 253 - 281) and Ebert and Creutzfeld, Diabetes Metab. Rev. 3, (1987) did not displace binding. Glucagon could displace the binding by 50 percent but only at a concentration of one micromolar (Fig. 4). The addition of subnanomolar concentrations of GLP-1 to transfected COS cells stimulated the production of cyclic AMP indicating that the receptor was functionally coupled to activation of adenylate cyclase (Fig. 5).

DNA sequence analysis of the rat GLP-1 receptor cDNA revealed a major open reading frame coding for a 463 amino acid polypeptide (SEQ ID No. 1). Hydrophaphy plot analysis indicated the presence of an amino-terminal hydrophobic region most probably representing a leader sequence. This hydrophobic segment is followed by a hydrophilic domain of about 120 amino

acids which contains three N-linked glycosylation sites. Seven hydrophobic segments are present which may form transmembrane domains. Search for sequence identities showed the GLP-1 receptor to be homologous to the secretin receptor (Ishihara et al. EMBO J. 10 (1991), 1635) (40 percent identity), the parathyroid hormone receptor (Jüppner et al. (Science 254 (1991), 1024) (32.4 percent identity) and the calcitonin receptor (Lin et al. Science 254 (1991), 1022) (27.5 percent identity) (Fig. 1). These four receptors do not share any significant sequence homology with other known members of the G-coupled receptor family and are characterized by a relatively long amino terminal, probably extracellular, domain. The sequence of the extracellular domain is unique for each receptor, yet four cysteines are perfectly conserved (boxes in Fig. 1). A fifth cysteine at position 126 of the GLP-1 receptor is also conserved in the parathyroid and calcitonin receptors and at a similar location in the secretin receptor (position 123). The highest sequence identity between the four proteins resides in the transmembrane domains. The carboxyl terminal, cytoplasmic, ends of each receptor are also very different. These receptors all stimulate the production of cyclic AMP in response to ligand binding (Ishihara et al. EMBO J. 10 (1991), 1635), Jüppner et al. (Science 254 (1991), 1024) and Lin et al. Science 254 (1991), 1022) and are presumably coupled to the cyclase via Gsα. In that respect, it is interesting to note that a sequence motif present in the third cytoplasmic loop of the GLP-1 receptors (RLAK, present just before the sixth transmembrane domain) is very similar to a motif of the beta2 adrenergic receptor (KALK) present at the same location and whose basic amino acids have been shown to be important in the coupling of the receptor to Gsα (Okamoto et al. Cell 67 (1991), 723). Moreover, in the beta2 adrenergic receptor, this motif is preceeded by a basic amino acid located twelve amino acid toward the amino-terminal end. This basic amino acid is also required at this particular distance for efficient coupling to Gsα. In the GLP-1 receptor a lysine residue is also present at

a similar location. This suggests that, despite the very low overall sequence identity, a structural feature may have been conserved in the third cytoplasmic loop between the two receptors which, may be required for the coupling of receptor to the Gsα protein.

Determination of the tissue distribution of the GLP-1 receptor was performed by Northern blot analysis. Northern blot analysis was performed with 10 µg of total RNA (Chomczynski and Sacchi, Anal.Biochem. 126 (1987), 156) denatured with glyoxal (McMaster and Carmichael, Proc.Natl.Acad.Sci. USA 74 (1977), 4835) separated on a 1% agarose gel and transferred to Nylon membranes (Thomas, Proc.Natl.Acad.Sci. USA 77 (1980), 5201). Hybridization was performed with the random primed labelled (Feinberg and Vogelstein, Anal.Biochem. 132 (1983), 6) 1,6 kb pGLPR-1 insert. Two mRNAs of 2.7 and 3.6 kb could be detected in pancreatic islets as well as in rat insulinoma cell lines (INS-1), in stomach and in lung (Fig. 6). No GLP-1 receptor mRNA could be detected in brain, liver, thymus, muscle, intestine and colon. The presence of the GLP-1 receptor has been reported in stomach where the peptide inhibits acid secretion by parietal cells in in vivo experiments (Schjoldager et al. Dig.Dis.Sci. 34 (1989), 703) but stimulates acid secretion on isolated parietal glands (Schmidtler et al. Am.J.Physiol. 260 (1991), G940). Binding sites for GLP-1 have also ben reported in lung membrane preparations (Richter et al. FEBS Letter 1 (1990), 78) but the role of the hormone on lung physiology is not known.

A stable cell line expressing the cloned rat GLP-1 receptor was established by Ca-phosphate mediated transfection (Maniatis et al., Molecular Cloning. A Laboratory Manual. Cold Spring Harbour Laboratory, 1989) of the CHL cell line (ATCC CCL39). The plasmid, pGLPR-1, which contains a 1.6 kb rat GLP-1 receptor cDNA insert cloned in the pCDNA-1 vector, was cotransfected with the pWL-neo plasmid (Stratagene, La Jolla,

CA) into CHL cells. The pWL-neo plasmid contains the neomycin resistance gene. Stable clones were selected in medium containing 0.8 mg/ml G418. A stable transformant expressing an estimate of 70.000 rat GLP-1 receptors pr cell was selected by this scheme and further propagated in the presence of 80 μM G418. Membranes from this transformant was subsequently used in the high-volume-throughput-screening (HVTS) assay as described in Example 3. Characterization of the receptor expressed by the GLP-1 R/CHL cell line led to an estimated Kd of 0.8 nM for whole cells, 2.3 nM for cell membranes using ^{125}I-GLP-1(7-36)amide as radioligand.

EXAMPLE 2

Molecular cloning of the human islet GLP-1 receptor cDNA.

Human islets were prepared as described (Ricordi et al., Diabetes 37 (1988), 413 - 420), and poly-A$^+$ RNA was isolated by affinity chromatography by published methods (Gonda et al., Mol. Cell. Biol. 2 (1982) 617 - 624).

A human islet cDNA library was constructed in the λZAPII vector from Stratagene (La Jolla, CA). Briefly, double stranded cDNA was synthesized as previously described (Aruffo and Seed, 84 (1987), 8573 - 8577; Thorens, Proc. Natl. Acad. Sci., USA 89 (1992), 8641 - 8645), and EcoRI/NotI adaptors (Stratagene, La Jolla, CA) were added with T_4 DNA ligase.

The resulting cDNA molecules were phosphorylated with T_4 polynucleotide kinase before size fractionation on potassium acetate gradients (Aruffo and Seed, 84 (1987), 8573 - 8577). Double stranded cDNA with a size above 1.6 kb was ligated into λZAPII arms (Stratagene, La Jolla, CA), packaged in λ phage and grown on a lawn of XL-1 Blue E. coli cells as described in protocols from Stratagene.

The cDNA library was screened by hybridization to a ^{32}P labelled

DNA fragment from the rat GLP-1 receptor cDNA by previously described methods (Maniatis et al., Molecular Cloning. A Laboratory Manual. Cold Spring Harbour Laboratory, 1982). The reduced stringency conditions used were: prehybridization and hybridization in 30 % formamide, 5 * SSC, 5 * Denhardt, 50 mM phosphate buffer pH 6.8, 5 mM EDTA, 0.2 % SDS and 100 µg/ml salmon sperm DNA at 42°C. Washings were 4 * 30 min in 2 * SSC, 0.2 % SDS at 42°C (Maniatis et al., Molecular Cloning. A Laboratory Manual. Cold Spring Harbour Laboratory, 1982).

Positive λ phages were purified by replating and hybridization, the cDNA inserts contained in the Bluescript vector present in the λ ZAPII arms were excised using helper phages obtained from Stratagene (La Jolla, CA). The inserts were partially sequenced. One clone designated 3(20) showed high homology to the rat GLP-1 receptor and was sequenced (Tabor and Richardson, Proc. Natl. Acad. Sci., USA 84 (1987), 4767 - 4771) in its entire length. The DNA sequence is shown as SEQ ID No. 3.

From homology analysis (Fig. 7), it was concluded that this cDNA encoded the 3' part of the human GLP-1 receptor. The deduced amino acid sequence of the human receptor has 92 % identity to the rat GLP-1 receptor in the region from amino acid number 170 to amino acid number 463 (numbers refer to the rat sequence).

The isolated human GLP-1 cDNA does not contain the entire open reading frame at the 5' end. However, a full length clone can easily be obtained by methods well known to persons skilled in the art. Among the alternative methods of choice, the following examples should be mentioned: 1) The human islet cDNA library can either be rescreened with a probe from the 5' end of the already cloned sequence. 2) Anchor-PCR or RACE (Rapid Amplification of cDNA Ends) (Kriangkum et al., Nucleic Acids Res. 20 (1992) 3793 - 3794; Troutt et al., Proc. Natl. Acad. Sci., USA 89 (1992), 9823 - 9825) methodology can be used to

clone the remaining 5' sequences from islet RNA. 3) The remaining 5' part can be isolated from human genomic libraries, and DNA fragments considered to represent introns can be identified based on homology to the cDNA of the rat receptor and deleted by mutagenesis.

After cloning of the 5' end of the open reading frame, this part of the cDNA can be fused to the remaining 3' part of the human GLP-1 receptor cDNA by the use of PCR or through fusion at appropriate restriction enzyme recognition sequences identified in both the 5' and the 3' parts.

The cDNA encoding the full length open reading frame can be cloned in suitable mammalian expression vectors and transfected into mammalian cell lines for expression. Examples of such suitable cell lines are the CHO and CHL cells, but other mammalian cells will also express receptors of this type.

It has recently been demonstrated that insect cells (Vasudevan et al. FEBS Lett. 311 (1992), 7 - 11) and microorganisms like e.g. yeast (King et al., Science 250 (1990), 121 - 123) can express G-protein coupled receptors.

Recently frog skin melanophore cells have been used to express G-protein coupled receptors (Potenza et al, Analytical Biochem., 206, (1992), 315 - 322) and a functional coupling to adenylate cyclase was demonstrated.

Other microorganisms like Aspergillus, Bacillus, E. coli might be able to express these receptors after appropriate genetic engineering and selection.

It is therefore clear to persons skilled in the art that a number of different expression systems can be designed that will lead to expression of a functional receptor molecule.

As demonstrated in Example 3, the rat as well as the human GLP-1 receptor can be used in screening assays for detection of new potential agonist lead structures.

EXAMPLE 3

High throughput screening assay for GLP-1 receptor agonists.

Screening of microbial extracts for secondary metabolites with potential GLP-1 agonist activity was carried out using the SPA (Scintillation Proximity Assay) technology (US patent 4568649, Hart and Greenwalt (Mol.Immunol., 16 (1979) 265-267), Udenfriend et al (Proc.Natl.Acad.Sci. USA, 82 (1985) 8672-8676). Wheatgerm agglutinin (WGA) coated SPA beads developed by Amersham International were used (US. patent 4568649, European patent 0154734, Japanese patent appl. 84/52452). The WGA coat allows GLP-1 receptor bearing membranes to be immobilized on the SPA beads. Membranes used in the screening assay were prepared from a CHL (ATTC CCL39) cell line expressing the cloned rat GLP-1 receptor as described in in Example 1. Membranes were prepared essentially as decribed by Unden et al (Eur.J.Biochem. 145 (1984), 525-530). The binding of ^{125}I-GLP-1(7-36)amide to such immobilized receptors brings the tracer in close proximity to the scintillant present within the SPA beads resulting in the emission of light. Any unbound ligand will not generate a signal. Thus under assay conditions a microbial extract - containing a component capable of binding to the GLP-1 receptor and thereby displacing the tracer - may be identified by virtue of a reduction in signal intensity.

A high throughput assay was established using 96 well microtiter plates. The assay was optimized with regard to the amounts of WGA particles, membrane and tracer used. (The ^{125}I-GLP-1(7-36)amide tracer was labelled using the lactoperoxidase method (Morrison et al., Methods Enzymol. 70 (1980), 214-219) followed by purification on reverse phase HPLC). Using a Pac-

kard TopCount™ microplate scintillation counter (Packard Instrument Company) these optimized conditions resulted in a B_0 of more than 7000 cpm. (Non specific binding determined in the presence of 500 nM unlabelled GLP-1(7-36)amide amounts to less than 1000 cpm. IC_{50}=0.5-1.0 nM GLP-1(7-36) amide).

So far 1250 microbial extracts have been screened using the SPA GLP-1 receptor assay. The extracts were tested at a final dilution of 1:400. Under these conditions 15 out of the 1250 extracts resulted in a reduction of specific counts to below the chosen cut-off level. These 15 hits have been further characterized in a secondary assay. This secondary assay was designed to test whether cAMP synthesis in a GLP-1 receptor bearing cell line can be induced by components in the extract. β-TC3 cells (Hanahan et al., Nature 315 (1985) 115-122) and Efrat et al (Proc.Natl.Acad.Sci. USA 85 (1988) 9037-9041) grown in 96-well microtiter plates were exposed to extracts diluted in culture media. After 20 min at 37°C the cells were lysed by addition of acid and the cAMP concentration determined using the cyclic AMP SPA system (Amersham International). Of the 15 primary hits tested in this secondary assay, 5 extracts have been found to significantly increase the cAMP level in β-TC3 cells.

It has thus been demonstrated that it is feasible that the screening approach described in this patent application can result in the isolation of natural compounds with GLP-1 agonist activity. The use of such compunds as lead structures for a medicinal chemistry approach will be of significant importance in the design of novel GLP-1 agonists.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Thorens, Bernard

(ii) TITLE OF INVENTION: Novel Peptide

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: NOVO NORDISK A/S, Patent Department
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(C) CITY: Bagsvaerd
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(F) ZIP: DK-2880

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: +45 44 44 88 88
(B) TELEFAX: +45 44 49 32 56
(C) TELEX: 37307

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3066 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rat

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 17..1408

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

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                    1               5                  10

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Leu Leu Leu Leu Gly Ala Val Gly Arg Ala Gly Pro Arg Pro Gln Gly
            15                  20                  25

GCC ACG GTG TCC CTC TCA GAG ACA GTG CAG AAA TGG AGA GAG TAT CGG       145
Ala Thr Val Ser Leu Ser Glu Thr Val Gln Lys Trp Arg Glu Tyr Arg
        30                  35                  40

CAC CAG TGC CAA CGT TTC CTC ACG GAA GCG CCA CTC CTG GCC ACA GGT       193
His Gln Cys Gln Arg Phe Leu Thr Glu Ala Pro Leu Leu Ala Thr Gly
    45                  50                  55
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CTC TTC TGC AAC CGA ACC TTT GAT GAC TAC GCC TGC TGG CCA GAT GGG      241
Leu Phe Cys Asn Arg Thr Phe Asp Asp Tyr Ala Cys Trp Pro Asp Gly
 60                  65                  70                  75

CCC CCA GGT TCC TTT GTG AAT GTC AGT TGC CCC TGG TAC CTG CCG TGG      289
Pro Pro Gly Ser Phe Val Asn Val Ser Cys Pro Trp Tyr Leu Pro Trp
                 80                  85                  90

GCC AGT AGT GTG CTC CAA GGG CAT GTG TAC CGG TTC TGC ACG GCC GAG      337
Ala Ser Ser Val Leu Gln Gly His Val Tyr Arg Phe Cys Thr Ala Glu
             95                  100                 105

GGT ATC TGG CTG CAT AAG GAC AAC TCC AGC CTG CCC TGG AGG GAC CTG      385
Gly Ile Trp Leu His Lys Asp Asn Ser Ser Leu Pro Trp Arg Asp Leu
         110                 115                 120

TCG GAG TGC GAA GAG TCC AAG CAA GGA GAG AGA AAC TCC CCT GAG GAA      433
Ser Glu Cys Glu Glu Ser Lys Gln Gly Glu Arg Asn Ser Pro Glu Glu
     125                 130                 135

CAG CTC CTG TCG CTG TAC ATT ATC TAC ACG GTG GGG TAC GCA CTT TCT      481
Gln Leu Leu Ser Leu Tyr Ile Ile Tyr Thr Val Gly Tyr Ala Leu Ser
140                 145                 150                 155

TTC TCT GCC TTG GTC ATC GCT TCA GCC ATC CTT GTC AGC TTC AGA CAC      529
Phe Ser Ala Leu Val Ile Ala Ser Ala Ile Leu Val Ser Phe Arg His
                160                 165                 170

TTG CAC TGC ACC AGG AAC TAC ATC CAC CTG AAC CTG TTT GCG TCC TTC      577
Leu His Cys Thr Arg Asn Tyr Ile His Leu Asn Leu Phe Ala Ser Phe
             175                 180                 185

ATC CTC CGA GCA CTG TCC GTC TTC ATC AAA GAC GCT GCC CTC AAG TGG      625
Ile Leu Arg Ala Leu Ser Val Phe Ile Lys Asp Ala Ala Leu Lys Trp
         190                 195                 200
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ATG TAT AGC ACG GCT GCG CAA CAG CAC CAG TGG GAT GGG CTC CTC TCG      673
Met Tyr Ser Thr Ala Ala Gln Gln His Gln Trp Asp Gly Leu Leu Ser
    205                 210                 215

TAT CAG GAC TCT CTG GGC TGC CGA CTG GTG TTC CTG CTC ATG CAA TAC      721
Tyr Gln Asp Ser Leu Gly Cys Arg Leu Val Phe Leu Leu Met Gln Tyr
220                 225                 230                 235

TGC GTG GCG GCC AAC TAC TAC TGG TTG CTG GTG GAA GGC GTG TAT CTG      769
Cys Val Ala Ala Asn Tyr Tyr Trp Leu Leu Val Glu Gly Val Tyr Leu
                240                 245                 250

TAC ACA CTG CTG GCC TTC TCG GTG TTC TCG GAG CAG CGC ATC TTC AAG      817
Tyr Thr Leu Leu Ala Phe Ser Val Phe Ser Glu Gln Arg Ile Phe Lys
            255                 260                 265

CTG TAC CTG AGC ATA GGC TGG GGA GTT CCG CTG CTG TTC GTT ATC CCC      865
Leu Tyr Leu Ser Ile Gly Trp Gly Val Pro Leu Leu Phe Val Ile Pro
        270                 275                 280

TGG GGC ATT GTC AAG TAT CTC TAC GAG GAC GAG GGT TGC TGG ACC AGG      913
Trp Gly Ile Val Lys Tyr Leu Tyr Glu Asp Glu Gly Cys Trp Thr Arg
    285                 290                 295

AAC TCC AAC ATG AAC TAT TGG CTC ATC ATA CGC TTG CCC ATT CTC TTT      961
Asn Ser Asn Met Asn Tyr Trp Leu Ile Ile Arg Leu Pro Ile Leu Phe
300                 305                 310                 315

GCA ATC GGG GTC AAC TTC CTT GTC TTC ATC CGG GTC ATC TGC ATC GTG     1009
Ala Ile Gly Val Asn Phe Leu Val Phe Ile Arg Val Ile Cys Ile Val
                320                 325                 330
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ATA GCC AAG CTG AAG GCT AAT CTC ATG TGT AAG ACC GAC ATC AAA TGC      1057
Ile Ala Lys Leu Lys Ala Asn Leu Met Cys Lys Thr Asp Ile Lys Cys
          335                 340                 345

AGA CTC GCG AAG TCC ACT CTG ACG CTC ATC CCG CTT CTG GGC ACG CAT      1105
Arg Leu Ala Lys Ser Thr Leu Thr Leu Ile Pro Leu Leu Gly Thr His
      350                 355                 360

GAA GTC ATC TTT GCC TTT GTG ATG GAC GAG CAC GCC CGA GGA ACC CTA      1153
Glu Val Ile Phe Ala Phe Val Met Asp Glu His Ala Arg Gly Thr Leu
  365                 370                 375

CGC TTC GTC AAG CTG TTC ACA GAG CTC TCC TTC ACT TCC TTC CAG GGC      1201
Arg Phe Val Lys Leu Phe Thr Glu Leu Ser Phe Thr Ser Phe Gln Gly
380                 385                 390                 395

TTT ATG GTG GCT GTC TTG TAC TGC TTT GTC AAC AAT GAG GTC CAG ATG      1249
Phe Met Val Ala Val Leu Tyr Cys Phe Val Asn Asn Glu Val Gln Met
            400                 405                 410

GAG TTT CGG AAG AGC TGG GAG CGC TGG AGG CTG GAG CGC TTG AAC ATC      1297
Glu Phe Arg Lys Ser Trp Glu Arg Trp Arg Leu Glu Arg Leu Asn Ile
          415                 420                 425

CAG AGG GAC AGC AGC ATG AAA CCC CTC AAG TGT CCC ACC AGC AGC GTC      1345
Gln Arg Asp Ser Ser Met Lys Pro Leu Lys Cys Pro Thr Ser Ser Val
      430                 435                 440

AGC AGT GGG GCC ACG GTG GGC AGC AGC GTG TAT GCA GCC ACC TGC CAA      1393
Ser Ser Gly Ala Thr Val Gly Ser Ser Val Tyr Ala Ala Thr Cys Gln
  445                 450                 455

AAT TCC TGC AGC TGAGCCCCAG TGCTGCGCTT CCTGATGGTC CTTGCTGCTG          1445
Asn Ser Cys Ser
460
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GCTGGGTGGC CATCCCAGGT GGGAGAGACC CTGGGGACAG GGAATATGAG GGATACAGGC 1505

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GCTGCAAGGC CCTTGGGGTT CCCTCGGACA GAACTGCAAA TCAACCCCGG AACTCTCGCT 1805

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CCTGCCCTTG GAACCTCAGC CAGCACAGCG CTCATCAAGG TGAGCTGGCT GTGCTGTGTG 1925

CACGGCTGGG GTTGTCACCT ACATCAGCCT TCCTCTCGGA CAAGAGGCTT TTCTCTGTGC 1985

ATCTGGAGTG CCGCCATTCC TCCATCTGCC CGTTCATCCG CCATCCTGTC TTTGCCTTGG 2045

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GCCTGGTCCT TTAAGCCACT TTGCTGCTTG GTTTCTGCCC TGCATGGGTA CTACTAGGGC 2225

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CTCAAATACC CTCTAAGGTT CATCTAAAGG AGTAACCTGC CTAGGGGTGC TGTTGACCTG 2345

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TGTGACCAGA GTCTATGCTT TGAATCAGAA TGGGCTATAT CCTCTGACCT CAGAGGCTAT 2465

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CACTGTGCCC CTTGGAGAGG CATAAGGCAT GTATGGGAGA TAATAATGGG CTATAAAACA 3065

T 3066

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 463 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Met Ala Val Thr Pro Ser Leu Leu Arg Leu Ala Leu Leu Leu Leu Gly
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Ala Val Gly Arg Ala Gly Pro Arg Pro Gln Gly Ala Thr Val Ser Leu
20 25 30

Ser Glu Thr Val Gln Lys Trp Arg Glu Tyr Arg His Gln Cys Gln Arg
35 40 45

Phe Leu Thr Glu Ala Pro Leu Leu Ala Thr Gly Leu Phe Cys Asn Arg
50 55 60

Thr Phe Asp Asp Tyr Ala Cys Trp Pro Asp Gly Pro Pro Gly Ser Phe
65 70 75 80

Val Asn Val Ser Cys Pro Trp Tyr Leu Pro Trp Ala Ser Ser Val Leu
85 90 95

Gln Gly His Val Tyr Arg Phe Cys Thr Ala Glu Gly Ile Trp Leu His
100 105 110

Lys Asp Asn Ser Ser Leu Pro Trp Arg Asp Leu Ser Glu Cys Glu Glu
115 120 125

Ser Lys Gln Gly Glu Arg Asn Ser Pro Glu Glu Gln Leu Leu Ser Leu
130 135 140

Tyr Ile Ile Tyr Thr Val Gly Tyr Ala Leu Ser Phe Ser Ala Leu Val
145 150 155 160

Ile Ala Ser Ala Ile Leu Val Ser Phe Arg His Leu His Cys Thr Arg
165 170 175

Asn Tyr Ile His Leu Asn Leu Phe Ala Ser Phe Ile Leu Arg Ala Leu
180 185 190

Ser Val Phe Ile Lys Asp Ala Ala Leu Lys Trp Met Tyr Ser Thr Ala
195 200 205

Ala Gln Gln His Gln Trp Asp Gly Leu Leu Ser Tyr Gln Asp Ser Leu
210 215 220

Gly Cys Arg Leu Val Phe Leu Leu Met Gln Tyr Cys Val Ala Ala Asn
225 230 235 240

Tyr Tyr Trp Leu Leu Val Glu Gly Val Tyr Leu Tyr Thr Leu Leu Ala
245 250 255

Phe Ser Val Phe Ser Glu Gln Arg Ile Phe Lys Leu Tyr Leu Ser Ile
260 265 270

Gly Trp Gly Val Pro Leu Leu Phe Val Ile Pro Trp Gly Ile Val Lys
275 280 285

Tyr Leu Tyr Glu Asp Glu Gly Cys Trp Thr Arg Asn Ser Asn Met Asn
290 295 300

Tyr Trp Leu Ile Ile Arg Leu Pro Ile Leu Phe Ala Ile Gly Val Asn
305 310 315 320

Phe Leu Val Phe Ile Arg Val Ile Cys Ile Val Ile Ala Lys Leu Lys
325 330 335

Ala Asn Leu Met Cys Lys Thr Asp Ile Lys Cys Arg Leu Ala Lys Ser
340 345 350

Thr Leu Thr Leu Ile Pro Leu Leu Gly Thr His Glu Val Ile Phe Ala
355 360 365

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370 375 380

Phe Thr Glu Leu Ser Phe Thr Ser Phe Gln Gly Phe Met Val Ala Val
385 390 395 400

Leu Tyr Cys Phe Val Asn Asn Glu Val Gln Met Glu Phe Arg Lys Ser
405 410 415

Trp Glu Arg Trp Arg Leu Glu Arg Leu Asn Ile Gln Arg Asp Ser Ser
420 425 430

Met Lys Pro Leu Lys Cys Pro Thr Ser Ser Val Ser Ser Gly Ala Thr
435 440 445

Val Gly Ser Ser Val Tyr Ala Ala Thr Cys Gln Asn Ser Cys Ser
450 455 460

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1909 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..887

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

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    1               5                  10                  15

GCA TCC TTC ATC CTG CGA GCA TTG TCC GTC TTC ATC AAG GAC GCA GCC          95
Ala Ser Phe Ile Leu Arg Ala Leu Ser Val Phe Ile Lys Asp Ala Ala
                  20                  25                  30

CTG AAG TGG ATG TAT AGC ACA GCC GCC CAG CAG CAC CAG TGG GAT GGG         143
Leu Lys Trp Met Tyr Ser Thr Ala Ala Gln Gln His Gln Trp Asp Gly
             35                  40                  45

CTC CTC TCC TAC CAG GAC TCT CTG AGC TGC CGC CTG GTG TTT CTG CTC         191
Leu Leu Ser Tyr Gln Asp Ser Leu Ser Cys Arg Leu Val Phe Leu Leu
         50                  55                  60

ATG CAG TAC TGT GTG GCG GCC AAT TAC TAC TGG CTC TTG GTG GAG GGC         239
Met Gln Tyr Cys Val Ala Ala Asn Tyr Tyr Trp Leu Leu Val Glu Gly
     65                  70                  75

GTG TAC CTG TAC ACA CTG CTG GCC TTC TCG GTG TTC TCT GAG CAA TGG         287
Val Tyr Leu Tyr Thr Leu Leu Ala Phe Ser Val Phe Ser Glu Gln Trp
 80                  85                  90                  95

ATC TTC AGG CTC TAC GTG AGC ATA GGC TGG GGT GTT CCC CTG CTG TTT         335
Ile Phe Arg Leu Tyr Val Ser Ile Gly Trp Gly Val Pro Leu Leu Phe
                100                 105                 110

GTT GTC CCC TGG GGC ATT GTC AAG ATC CTC TAT GAG GAC GAG GGC TGC         383
Val Val Pro Trp Gly Ile Val Lys Ile Leu Tyr Glu Asp Glu Gly Cys
            115                 120                 125

TGG ACC AGG AAC TCC AAC ATG AAC TAC TGG CTC ATT ATC CGG CTG CCC         431
Trp Thr Arg Asn Ser Asn Met Asn Tyr Trp Leu Ile Ile Arg Leu Pro
        130                 135                 140
```

```
ATT CTC TTT GCC ATT GGG GTG AAC TTC CTC ATC TTT GTT CGG GTC ATC      479
Ile Leu Phe Ala Ile Gly Val Asn Phe Leu Ile Phe Val Arg Val Ile
    145                 150                 155

TGC ATC GTG GTA TCC AAA CTG AAG GCC AAT GTC ATG TGC AAG ACA GAC      527
Cys Ile Val Val Ser Lys Leu Lys Ala Asn Val Met Cys Lys Thr Asp
160                 165                 170                 175

ATC AAA TGC AGA CTT GCC AAG TCC ACG CTG ACA CTC ATC CCC CTG CTG      575
Ile Lys Cys Arg Leu Ala Lys Ser Thr Leu Thr Leu Ile Pro Leu Leu
                180                 185                 190

GGG ACT CAT GAG GTC ATC TTT GCC TTT GTG ATG GAC GAG CAC GCC CGG      623
Gly Thr His Glu Val Ile Phe Ala Phe Val Met Asp Glu His Ala Arg
            195                 200                 205

GGG ACC CTG CGC TTC ATC AAG CTG TTT ACA GAG CTC TCC TTC ACC TCC      671
Gly Thr Leu Arg Phe Ile Lys Leu Phe Thr Glu Leu Ser Phe Thr Ser
        210                 215                 220

TTC CAG GGG CTG ATG GTG GCC ATC TTA TAC TGC TTT GTC AAC AAT GAG      719
Phe Gln Gly Leu Met Val Ala Ile Leu Tyr Cys Phe Val Asn Asn Glu
    225                 230                 235

GTC CAG CTG GAA TTT CGG AAG AGC TGG GAG CGC TGG CGG CTT GAG CAC      767
Val Gln Leu Glu Phe Arg Lys Ser Trp Glu Arg Trp Arg Leu Glu His
240                 245                 250                 255

TTG CAC ATC CAG AGG GAC AGC AGC ATG AAG CCC CTC AAG TGT CCC ACC      815
Leu His Ile Gln Arg Asp Ser Ser Met Lys Pro Leu Lys Cys Pro Thr
                260                 265                 270

AGC AGC CTG AGC AGT GGA GCC ACG GCG GGC AGC AGC ATG TAC ACA GCC      863
Ser Ser Leu Ser Ser Gly Ala Thr Ala Gly Ser Ser Met Tyr Thr Ala
            275                 280                 285
```

ACT TGC CAG GCC TCC TGC AGC TGAGACTCCA GCGCCTGCCC TCCCTGGGGT 914
Thr Cys Gln Ala Ser Cys Ser
290 295

CCTTGCTGCG GCCGGGTGGC AATCCAGGAG AAGCAGCCTC CTAATTTGAT CACAGTGGCG 974

AGAGGAGAGG AAAAACGATC GCTGTGAAAA TGAGGAGGAT TGCTTCTTGT GAAACCACAG 1034

GCCCTTGGGG TTCCCCCAGA CAGAGCCGCA AATCAACCCC AGACTCAAAC TCAAGGTCAA 1094

CGGCTTATTA GTGAAACTGG GGCTTGCAAG AGGAGGTGGT TCTGAAAGTG GCTCTTCTAA 1154

CCTCAGCCAA ACACGAGCGG GAGTGACGGG AGCCTCCTCT GCTTGCATCA CTTGGGGTCA 1214

CCACCCTCCC CTGTCTTCTC TCAAAGGGAA GCTGTTTGTG TGTCTGGGTT GCTTATTTCC 1274

CTCATCTTGC CCCCTCATCT CACTGCCCAG TTTCTTTTTG AGGGCTTGTT GGCCACTGCC 1334

AGCAGCTGTT TCTGGAAATG GCTGTAGGTG GTGTTGAGAA AGAATGAGCA TTGAGACACG 1394

GTGCTCGCTT CTCCTCCAGG TATTTGAGTT GTTTTGGTGC CTGCCTCTGC CATGCCCAGA 1454

GAATCAGGGC AGGCTTGCCA CCGGGGAACC CAGCCCTGGG GTATGAGCTG CCAAGTCTAT 1514

TTTAAAGACG CTCAAGAATC CTCTGGGGTT CATCTAGGGA CACGTTAGGA ATGTCCAGAC 1574

TGTGGGTGTA GGTTACCTGC CACTTCCAGG ACGCAGAGGG CCAAGAGAGA CATTGCCTCC 1634

ACCTCTCCTG AATACTTATC TGTGACCACA CGCTGTCTCT TGAGATTTGG ATACACTCTC 1694

TAGCTTTAGG GGACCATGAA GAGACTCTCT TAGGAAACCA ATAGTCCCCA TCAGCACCAT 1754

GGAGGCAGGC TCCCCCTGCC TTTGAAATTC CCCCACTTGG GAGCTGATAT ACTTCACTCA 1814

CTTTTCTTTA TTGCTGTGAT AGTCTGTGTG CACAATGGGC AATTCTGACT TCTCCCATCT 1874

AGTGAAATGA GCGAAATCAT GGTTGTAGTG ATCTT 1909

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 294 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Arg His Leu Tyr Cys Thr Arg Asn Tyr Ile His Leu Asn Leu Phe Ala
1 5 10 15

Ser Phe Ile Leu Arg Ala Leu Ser Val Phe Ile Lys Asp Ala Ala Leu
20 25 30

Lys Trp Met Tyr Ser Thr Ala Ala Gln Gln His Gln Trp Asp Gly Leu
35 40 45

Leu Ser Tyr Gln Asp Ser Leu Ser Cys Arg Leu Val Phe Leu Leu Met
50 55 60

Gln Tyr Cys Val Ala Ala Asn Tyr Tyr Trp Leu Leu Val Glu Gly Val
65 70 75 80

Tyr Leu Tyr Thr Leu Leu Ala Phe Ser Val Phe Ser Glu Gln Trp Ile
85 90 95

Phe Arg Leu Tyr Val Ser Ile Gly Trp Gly Val Pro Leu Leu Phe Val
100 105 110

Val Pro Trp Gly Ile Val Lys Ile Leu Tyr Glu Asp Glu Gly Cys Trp
115 120 125

Thr Arg Asn Ser Asn Met Asn Tyr Trp Leu Ile Ile Arg Leu Pro Ile
130 135 140

Leu Phe Ala Ile Gly Val Asn Phe Leu Ile Phe Val Arg Val Ile Cys
145 150 155 160

Ile Val Val Ser Lys Leu Lys Ala Asn Val Met Cys Lys Thr Asp Ile
165 170 175

Lys Cys Arg Leu Ala Lys Ser Thr Leu Thr Leu Ile Pro Leu Leu Gly
180 185 190

Thr His Glu Val Ile Phe Ala Phe Val Met Asp Glu His Ala Arg Gly
195 200 205

Thr Leu Arg Phe Ile Lys Leu Phe Thr Glu Leu Ser Phe Thr Ser Phe
210 215 220

Gln Gly Leu Met Val Ala Ile Leu Tyr Cys Phe Val Asn Asn Glu Val
225 230 235 240

Gln Leu Glu Phe Arg Lys Ser Trp Glu Arg Trp Arg Leu Glu His Leu
245 250 255

His Ile Gln Arg Asp Ser Ser Met Lys Pro Leu Lys Cys Pro Thr Ser
260 265 270

Ser Leu Ser Ser Gly Ala Thr Ala Gly Ser Ser Met Tyr Thr Ala Thr
275 280 285

Cys Gln Ala Ser Cys Ser
290

CLAIMS

1. A recombinant glucagon-like peptide-1 (GLP-1) receptor.

2. A GLP-1 receptor according to claim 1 of mammalian origin.

3. A GLP-1 receptor according to claim 2 of rat or human origin.

4. A GLP-1 receptor according to claim 3, which comprises the amino acid sequence shown in SEQ ID No. 1, or an analogue thereof binding GLP-1 with an affinity constant below 100 nM, preferably below 10 nM.

5. A GLP-1 receptor according to claim 3, which comprises the partial amino acid sequence shown in SEQ ID No. 3, or an analogue thereof binding GLP-1 with an affinity constant below 100 nM, preferably below 10 nM.

6. A GLP-1 receptor according to any of the claims 1 to 5, which is in a solubilised or reconstituted form.

7. A DNA construct which comprises a DNA sequence encoding a GLP-1 receptor according to any of the claims 1 to 6.

8. A DNA construct according to claim 7, which comprises the DNA sequence shown in SEQ ID No. 1, or a DNA sequence coding for a functional analogue thereof binding GLP-1 with an affinity constant below 100 nM, preferably below 10 nM.

9. A DNA construct according to claim 7, which comprises the partial DNA sequence shown in SEQ ID No. 3, or a DNA sequence coding for a functional analogue thereof binding GLP-1 with an affinity constant below 100 nM, preferably below 10 nM.

10. A recombinant expression vector which carries an inserted DNA construct according to any of claims 7 to 9.

11. A cell containing a recombinant expression vector according to claim 10.

12. A cell containing a DNA construct according to any of claims 7 to 9 integrated in its genome.

13. A cell according to claim 11 or 12, which is an eukaryotic cell, in particular an insect or a mammalian cell.

14. A method of screening for agonists or enhancers of GLP-1 activity, the method comprising incubating a GLP-1 receptor according to any of claims 1 to 6 with a substance suspected to be an agonist of GLP-1 activity and subsequently with a GLP-1 or an analogue thereof, and detecting any effect of binding of GLP-1 or the analogue to the GLP-1 receptor.

15. A method of screening for agonists or enhancers of GLP-1 activity, the method comprising incubating GLP-1 or an analogue thereof with a substance suspected to be an agonist of GLP-1 activity and subsequently with a GLP-1 receptor of the invention, and detecting any effect of binding of GLP-1 or the analogue to the receptor.

16. Use of a GLP-1 receptor according to any of claims 1 to 6 for screening for agonists of GLP-1 activity.

17. Use of DNA constructs according to claims 7 to 9 for isolation of tissue and/or organ specific variants of the GLP-1 receptor.

18. Use of a receptor isolated according to claim 17 for the screening of GLP-1 agonists.

```
GLPR   MAVTP-----SLLR-----LALLLLGAVGRAGPRPQGA----------    28
SECR   MLSTMRPR-LSLLL-----LRLLLLTKAAHTV----GV----------    28
PTHR   MGAPRISHSLALLLCCSVLSSVYALVDADDVITKEEQIILLRNAQAQCEQEL    52
CTR1   MRFTLTRWCLTLFIFLNRPLPVLPDSADGAHTPTLEPEPFLY----------    42
       *  .      .*.         .       .

GLPR   -----TVSLSETVQKWREYRHQCQRFLTE----------APLLATGLF    61
SECR   -----PPRLCDVRRVLLEERAHCLQQLSKEK--------KGALGPETASG--    65
PTHR   KEVLRVPELAESAKDWMSRSAKTKKEKPAEKLYPQAEESREVSDRSRLQDGF   104
CTR1   --ILGKQRM-------LEAQHRC---------Y----DRMQKLPPYQGEGLY    72
              .        .   .                    .    .

       ↓                          ↓
GLPR   C NRTFDDYA C WPDGPPGSFVNVS C PWYLPWASSVLQGHVYRF C T    105
SECR   C EGLWDNMS C WPSSAPARTVEVR C PKSLLSLSNK-NGSLFRN C T    108
PTHR   C LPEWDNIV C WPAGVPGKVVAVP C PDYFYDFNHK--GRAYRR C D    146
CTR1   C NRTWDGWS C WDDTPAGVLAEQY C PDYFPDFDA--AEKVTKY C G    114
       *    .*.   * * .. .. ..   * *  .   .    .   .  * .

                ↓                                      I
GLPR   AEGIWLHKDNSSLPWRDLSECEESKQGERNSPEEQLLSLYIIYTVGYALSFS    157
SECR   QDG-W----SETFPRPDLACGVNINNSFNERRHAYLLKLKVMYTVGYSSSLA    155
PTHR   SNGSWELVPGNNRTWANYSECVKFLTNETREREV-FDRLGMIYTVGYSISLG    197
CTR1   EDGDWYRHPESNISWSNYTMCNAFTP--DKLQNAYI--LYYLAIVGHSLSIL    162
        .* *    ... .. . .             ..  .  *  . .**.. *.

                                    II
       ALVIASAILVSFRHLHCTRNYIHLNLFASFILRALSVFIKDAALKWMYSTAA    209
SECR   MLLVALSILCSFRRLHCTRNYIHMHLFVSFILRALSNFIKDAVL---FSSDD    204
PTHR   SLTVAVLILGYFRRLHCTRNYIHMHLFVSFMLRAVSIFIKDAVLYSGVSTDE    249
CTR1   TLLISLGIFMFLRSISCQRVTLHKNMFLTYVLNSIIIIVHLVVI--------    206
        * ..  *.  .* . * *  .* ..* ...*...  ... ...

                                        III
GLPR   QQHQWDG-LLSY--QDS------LGCRLVFLLMQYCVAANYYWLLVEGVYLY    252
SECR   ---------VTYCDAHK------VGCKLVMIFFQYCIMANYAWLLVEGLYLH    241
PTHR   IERITEEELRAFTEPPPADKAGFVGCRVAVTVFLYFLTTNYYWILVEGLYLH    301
CTR1   ---VPNGELVK-RDPPI--------CKVLHFFHQYMMSCNYFWMLCEGVYLH    246
                  .       *..      * .  ** *.* **.**.
```

Fig. 1A

```
                                IV
GLPR   TLLAFSVFSEQRIFKLYLSIGWGVPLLFVIPWGIVKYLYEDEGCWTRNSNMN   304
SECR   TLLAISFFSERKYLQAFVLLGWGSPAIFVALWAITRHFLENTGCWDINANAS   293
PTHR   SLIFMAFFSEKKYLWGFTLFGWGLPAVFVAVWVTVRATLANTECWDLSSGNK   353
CTR1   TLIVVSVFAEGQRLWWYHVLGWGFPLIPTTAHAITRANLFNDNCW-LSVDTN   297
       .*.  . *.* . .  .  .*** * . .    ...    ...**  . . .

                 V
GLPR   YWLIIRLPILFAIGVNFLVFIRVICIVIAKLKANLMCKTDIKC---RLAKST   353
SECR   VWWVIRGPVILSILINFIFFINILRILMRKLRTQETRGSETNH-YKRLAKST   344
PTHR   KW-IIQVPILAAIVVNFILFINIIRVLATKLRETNAGRCDTRQQYRKLLKST   404
CTR1   LLYIIHGPVMAALVVNFFFLLNILRVLVKKLKESQE---AESHMYLKAVRAT   346
          .*. *.. .. .**. ..... ..  **..      . .    .  ..*

          VI                                   VII
GLPR   LTLIPLLGTHEVIFAFVMDEHARGTLRFVKLFTELSFTSFQGFMVAVLYCFV   405
SECR   LLLIPLFGIHYIVFAFSHEDAME-----VQLFFELALGSFQGLVVAVLYCFL   391
PTHR   LVLMPLFGVHYIVFMATPYTEVSGILWQVQMHYEMLFNSFQGFFVAIIYCFC   456
CTR1   LILVPLLGVQFVVLPWRPSTPLLGKIYD---YVVHSLIHFQGFFVAIIYCFC   395
       * *.**.*.. ...     .                .  ***. **..***

GLPR   NNEVQMEFRKSWERWRLE-RLNIQRDSSMKPLKC------------------   438
SECR   NGEVQLEVQKKWRQWHLQ-EFPLRPVAFNNSFSN------------------   424
PTHR   NGEVQAEIKKSWSRWTLALDFKRKARSGSSTYSYGPMVSHTSVTNVGPRGGL   508
CTR1   NHEVQGALKRQWNQ------YQAQRWAGRRS----TRAANAAAATAAAAAAL   437
       * *** . .. * .          .  .  ..

GLPR   ------------------------------------PTSSVSSGATV-----   449
SECR   ------------------------------------ATNGPTHSTKA-----   435
PTHR   ALSLSPRLAPGAGASANGHHQLPGYVKHGSISENSLPSSGPEPGTKDDGYLN   560
CTR1   AETV----------------EIPVYICHQEPREEP---AGEEPVVEVEG---   467
                                                  .. .  ..

GLPR   GSSVYAATC-----------QNSCS   463
SECR   STEQSRSIP-----------RASII   449
PTHR   GSGLYEPMVGEQPPPLLEEERETVM   585
CTR1   --------VEVIAMEVLEQE--TSA   482
```

Fig. 1B

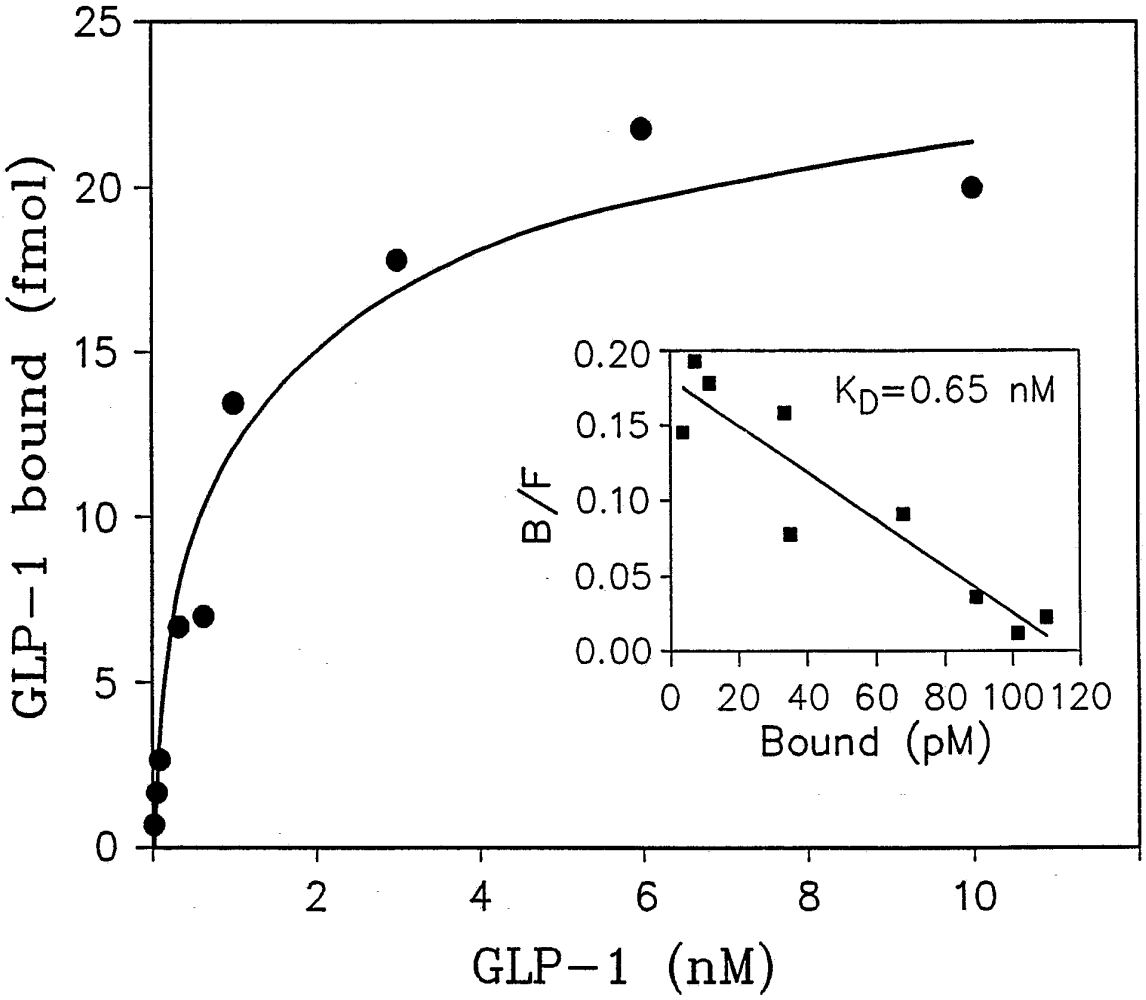
GLP-1 bound (fmol)
25
20
15
10
5
0
2
4
6
8
10
GLP-1 (nM)
B/F
0.20
0.15
0.10
0.05
0.00
K_D=0.65 nM
0
20
40
60
80
100
120
Bound (pM)

FIG. 2

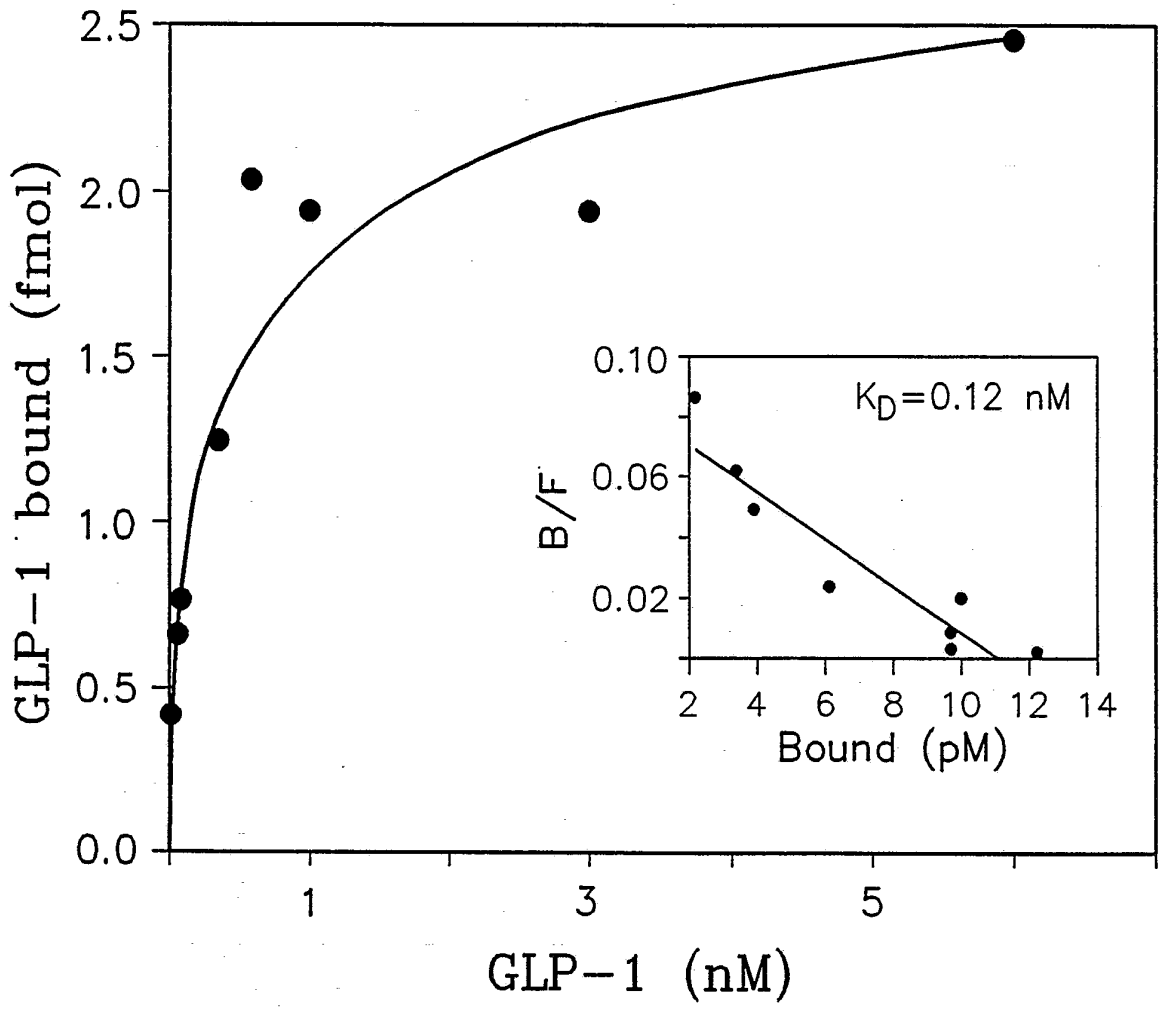
2.5
2.0
1.5
1.0
0.5
0.0
GLP-1 bound (fmol)
1
3
5
GLP-1 (nM)
0.10
0.06
0.02
B/F
K_D=0.12 nM
2
4
6
8
10
12
14
Bound (pM)

FIG. 3

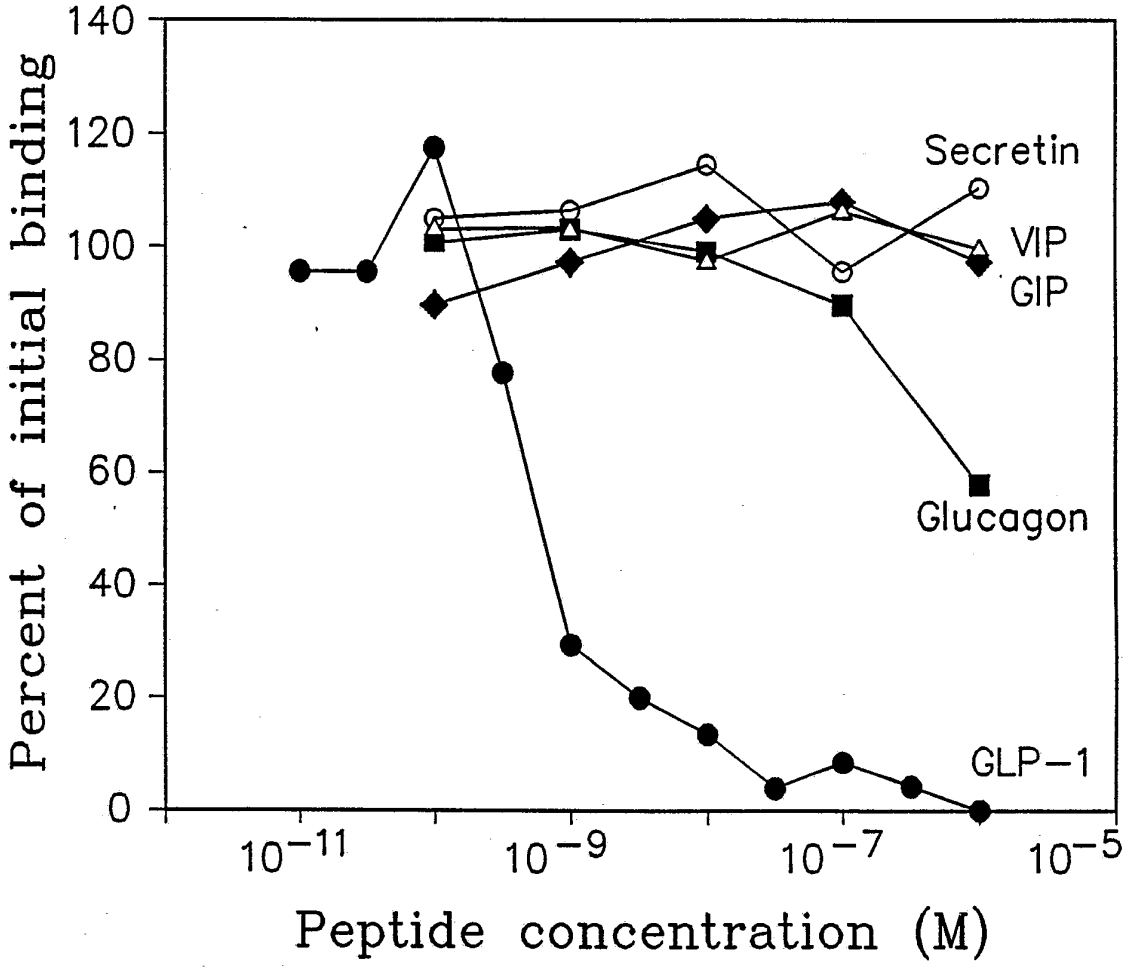
Percent of initial binding
140
120
100
80
60
40
20
0
Secretin
VIP
GIP
Glucagon
GLP-1
10^{-11}
10^{-9}
10^{-7}
10^{-5}
Peptide concentration (M)

FIG. 4

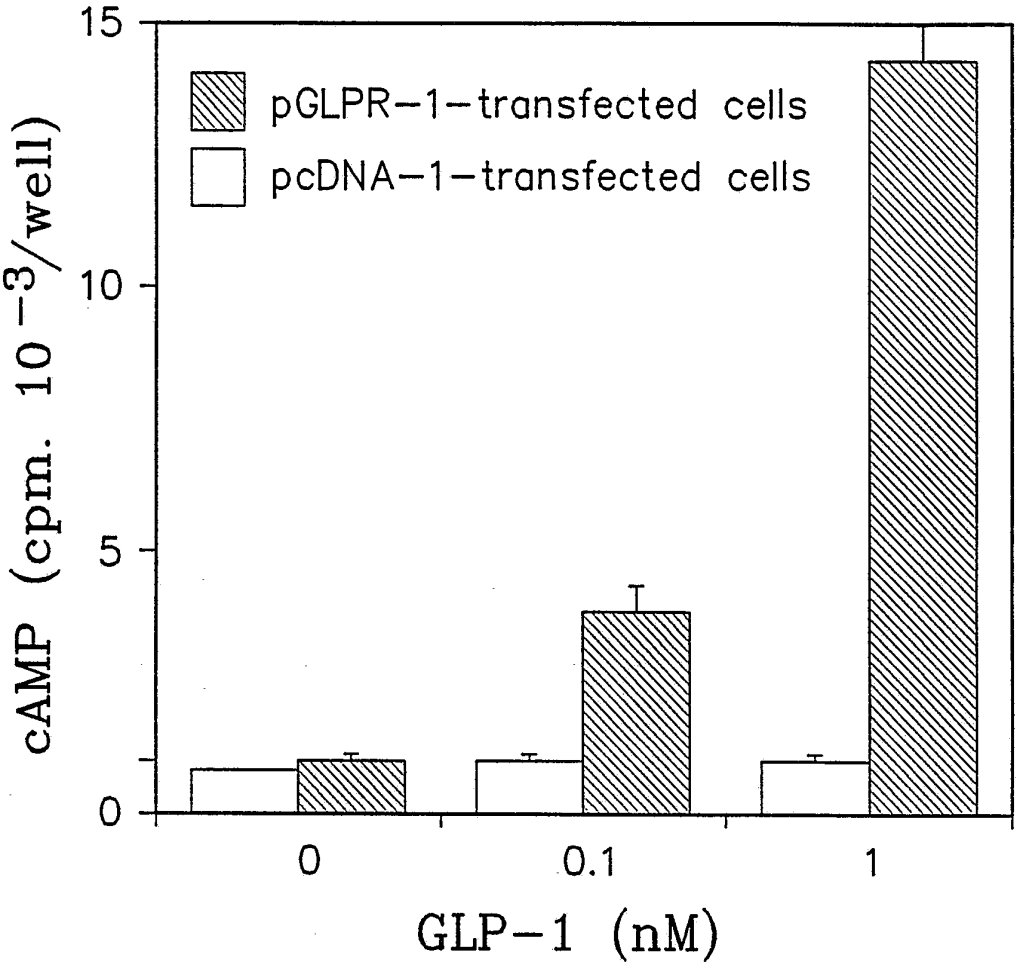
15
10
5
0
cAMP (cpm. 10^{-3}/well)
pGLPR-1-transfected cells
pcDNA-1-transfected cells
0
0.1
1
GLP-1 (nM)

FIG. 5

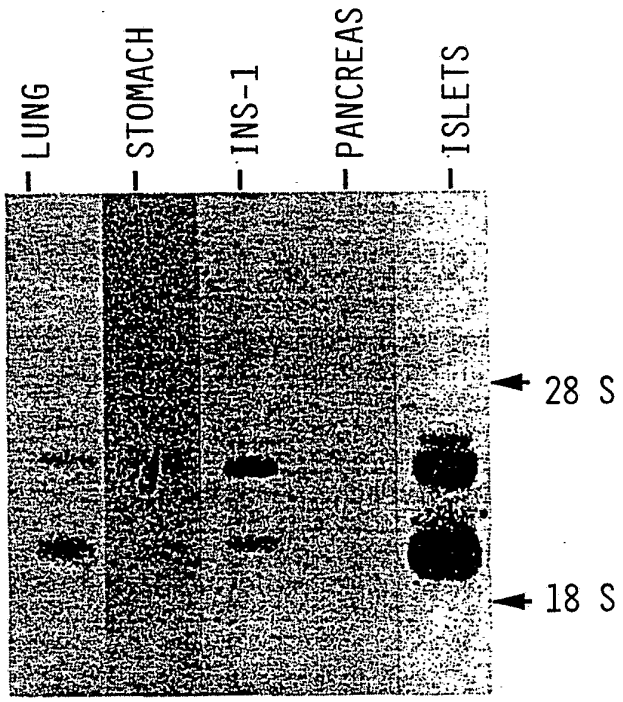

FIG. 6

```
RAT  - MAVTPSLLRLALLLLGAVGRAGPRPQGATVSLSETVQKWREYRHQCQRFL - 50

RAT  - TEAPLLATGLFCNRTFDDYACWPDGPPGSFVNVSCPWYLPWASSVLQGHV -100

RAT  - YRFCTAEGIWLHKDNSSLPWRDLSECEESKQGERNSPEEQLLSLYIIYTV -150

RAT  - GYALSFSALVIASAILVSFRHLHCTRNYIHLNLFASFILRALSVFIKDAA -200
                          ::: ::::::::::::::::::::::::::::
HUM  -                    RHLYCTRNYIHLNLFASFILRALSVFIKDAA - 31

RAT  - LKWMYSTAAQQHQWDGLLSYQDSLGCRLVFLLMQYCVAANYYWLLVEGVY -250
       :::::::::::::::::::::::: ::::::::::::::::::::::::::
HUM  - LKWMYSTAAQQHQWDGLLSYQDSLSCRLVFLLMQYCVAANYYWLLVEGVY - 81

RAT  - LYTLLAFSVFSEQRIFKLYLSIGWGVPLLFVIPWGIVKYLYEDEGCWTRN -300
       :::::::::::::: :: :: :::::::::::: :::::: ::::::::::
HUM  - LYTLLAFSVFSEQWIFRLYVSIGWGVPLLFVVPWGIVKILYEDEGCWTRN -131

RAT  - SNMNYWLIIRLPILFAIGVNFLVFIRVICIVIAKLKANLMCKTDIKCRLA -350
       ::::::::::::::::::::::: : ::::::  ::::::::::::::::::
HUM  - SNMNYWLIIRLPILFAIGVNFLIFVRVICIVVSKLKANLMCKTDIKCRLA -181

RAT  - KSTLTLIPLLGTHEVIFAFVMDEHARGTLRFVKLFTELSFTSFQGFMVAV -400
       :::::::::::::::::::::::::::::::: :::::::::::::: :::
HUM  - KSTLTLIPLLGTHEVIFAFVMDEHARGTLRFIKLFTELSFTSFQGLMVAI -231

RAT  - LYCFVNNEVQMEFRKSWERWRLERLNIQRDSSMKPLKCPTSSVSSGATVG -450
       :::::::::: :::::::::::: : ::::::::::::::: ::::: :
HUM  - LYCFVNNEVQLEFRKSWERWRLEHLHIQRDSSMKPLKCPTSSLSSGATAG -281

RAT  - SSVYAATCQNSCS -463
       :: : :::: :::
HUM  - SSMYTATCQASCS -294
```

FIG. 7

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 93/00697

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; C07K13/00; C12N5/10; G01N33/74

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C07K ; G01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 89, no. 18, 15 September 1992, WASHINGTON US pages 8641 - 8645 THORENS B; 'Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1.' see the whole document ---	1-18
A	FEBS LETTERS. vol. 267, no. 1, July 1990, AMSTERDAM NL pages 78 - 80 Richter G;Goke R;Goke B;Arnold R; 'Characterization of receptors for glucagon-like peptide-1(7-36)amide on rat lung membranes.' --- -/--	1-18

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 JULY 1993	27. 07. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NAUCHE S.A.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	FEBS LETTERS. vol. 262, no. 1, March 1990, AMSTERDAM NL pages 139 - 141 UTTENTHAL, L.O. ET AL; 'Characterization of high-affinity receptors for truncated glucagon-like peptide-1 in rat gastric glands.' see the whole document -----	1-18