Rapid Publications

Cloning and Functional Expression of the Human Islet GLP-1 Receptor

Demonstration That Exendin-4 Is an Agonist and Exendin-(9–39) an Antagonist of the Receptor

BERNARD THORENS, ANDRÉE PORRET, LÉO BÜHLER, SHAO-PING DENG, PHILIPPE MOREL, AND CHRISTIAN WIDMANN

A complementary DNA for a glucagon-like peptide-1 receptor was isolated from a human pancreatic islet cDNA library. The isolated clone encoded a protein with 90% identity to the rat receptor. In stably transfected fibroblasts, the receptor bound [^{125}I]GLP-1 with high affinity (K_d = 0.5 nM) and was coupled to adenylate cyclase as detected by a GLP-1–dependent increase in cAMP production (EC_{50} = 93 pM). Two peptides from the venom of the lizard *Heloderma suspectum*, exendin-4 and exendin-(9–39), displayed similar ligand binding affinities to the human GLP-1 receptor. Whereas exendin-4 acted as an agonist of the receptor, inducing cAMP formation, exendin-(9–39) was an antagonist of the receptor, inhibiting GLP-1–induced cAMP production. Because GLP-1 has been proposed as a potential agent for treatment of NIDDM, our present data will contribute to the characterization of the receptor binding site and the development of new agonists of this receptor. *Diabetes* 42:1678–82, 1993

In normal individuals, oral glucose absorption induces a greater insulin secretory response than an isoglycemic intravenous glucose infusion. This greater effect of oral glucose results from the secretion of intestinal hormones, which, at the level of the pancreatic β-cells, potentiate glucose-induced insulin secretion (1,2). These hormones are thus referred to as glucoincretins. The two main glucoincretins characterized so far are GIP and GLP-1 (3–5). GLP-1 is generated in intestinal L-cells by a cell-specific proteolytic cleavage of the preproglucagon molecule (6). This hormone is a glucoincretin in both rats and humans (1,3,7). Importantly, the stimulatory action of GLP-1 on insulin secretion requires the presence of glucose at normal or slightly elevated concentration and is mediated by elevation in intracellular cAMP (8,9). In addition to its stimulatory effect on secretion, GLP-1 also stimulates insulin gene transcription (9).

In diabetes, the total incretin effect is markedly reduced (1). This, however, does not result from a decreased secretion of GLP-1 or of GIP because normal or even elevated circulating levels of these hormones have been found in NIDDM patients (10–12). Therefore, the decreased incretin effect may be caused by a deficient action of these hormones at the β-cell level, either as a result of decreased receptor number or because of defects in intracellular signal transduction. However, recent studies have shown that infusion of pharmacological doses of GLP-1, but not of GIP, could ameliorate postprandial glucose levels by stimulating insulin secretion in diabetic patients (10,13,14). Because GLP-1 insulinotropic action is glucose dependent, this peptide or agonists of its receptor could be used as new antidiabetic agents without the risk of inducing hypoglycemia, a complication often encountered with the currently used antidiabetic sulfonylureas (15).

The possible therapeutic use in NIDDM of GLP-1 receptor agonists stresses the need for the characterization of the human GLP-1 receptor. We report herein the characterization by molecular cloning and functional expression of a human pancreatic islet's GLP-1 receptor. Moreover, we demonstrate that two peptides of related structure, exendin-4 and exendin-(9–39) (16), are agonists and antagonists of the receptor, respectively. This will help characterize the receptor binding site and may

From the Department of Pharmacology and Toxicology, University of Lausanne, Lausanne; Transplantation Unit, Department of Surgery, Geneva University Hospital, Geneva, Switzerland.

Address correspondence and reprint requests to Dr. Bernard Thorens, Institute of Pharmacology and Toxicology, 27 Bugnon, 1005 Lausanne, Switzerland.

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GLP-1, glucagon-like peptide-1(7,36)amide; NIDDM, non-insulin-dependent diabetes mellitus; GIP, gastric inhibitory peptide; kb, kilobase; SSC, sodium chloride-sodium citrate; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; IBMX, isobutylmethylxanthine; VIP, vasoactive intestinal peptide; K_d, dissociation constant; cpm, counts per minute.

be an important step in the design of new antidiabetic drugs acting through the GLP-1 receptor.

RESEARCH DESIGN AND METHODS

Library construction and screening. A human pancreatic islet cDNA library constructed in λ-gt11 (17) (gift of Mike M. Mueckler, Saint Louis, MO) was screened with a 1.12-kb *Kpn*I-*Pst*I fragment derived from the pGLPR-1 plasmid, which contains a full-length rat GLP-1 receptor cDNA (18). The probe was labeled by random priming (19) and hybridization was performed at reduced stringency: 30% formamide, 5 × SSC (1 × SSC: 150 mM NaCl, 15 mM Na3 citrate, pH 7.0), 0.2% SDS, 5 × Denhardt (1 × Denhardt: 0.2 g/L BSA, 0.2 g/L polyvinylpyrrolidone, 0.2 g/L Ficoll), 50 mM sodium phosphate, pH 6.8, 5 mM EDTA, and 100 ug/ml salmon sperm DNA, at 42°C for 18 h. Washings were done 4 times for 20 min in 2 × SSC, 0.2% SDS at 42°C. The filters were then exposed with an intensifier screen to Kodak XAR-5 films at −70°C. Positive clones were purified by two or three additional cycles of screening in the same conditions. A second human islet cDNA library was constructed as follows. Human islets purified by the method of Ricordi et al. (20,21) were used to prepare polyA RNA by the method of Gonda et al. (22). Double-stranded cDNAs were prepared from 4 μg of polyA RNA and size-selected according to Aruffo and Seed (23), as described previously (18). cDNA with sizes >1.6 kb were pooled and ligated in *Eco*RI-cut λ-ZAP II (Stratagene, La Jolla, CA). The ligation reaction was used for packaging with GigaPack Gold extracts (Stratagene). The phages were then grown on a lawn of XL1-Blue bacteria. Screening of the unamplified library (5.10^5 independent plaque forming units) was performed with the partial clone obtain from the λ-gt11 library as a probe. Inserts of plaque-purified phages were excised into Bluescript SK(−) by superinfection with the helper phage R408 (Stratagene), as per the manufacturer's protocol.

DNA sequencing and analysis. Sequencing of the cDNA inserts was performed on double-stranded DNA by the chain termination technique (24) with Sequenase (U.S. Biochemical, Cleveland, OH) using synthetic primers derived from the deduced nucleotide sequence and buffer gradient gel electrophoresis (25).

Expression of the human GLP-1 receptor. The cDNA clone encoding the complete GLP-1 receptor was subcloned into *Hin*dIII-*Bam*HI-cut pcDNA-1 expression vector (InVitrogen, San Diego, CA). Chinese hamster fibroblasts (CHL cells, ATCC CCL39) were cotransfected with the receptor cDNA in the pcDNA-1 vector and the pWL-neo vector (Stratagene) containing the neomycin resistance gene. Clones were selected in the presence of 0.8 mg/ml G418 (Geneticin, Gibco/BRL, Gaithersberg, MD). Iodinated GLP-1 was bound at 4°C for 15–18 h in Hank's balanced salt solution containing 20 mM HEPES, pH 7.4, 0.5% BSA, and 1 mM phenylmethylsulfonylfluoride. The cells were then washed 4 times with ice-cold saline solution, lysed in 0.2 N NaOH, 1% SDS, and the radioactivity was measured with a γ-counter. GLP-1 and the other peptides were from Peninsula (Belmont, CA) or Bachem (Torrence, CA) except exendin-4 and exendin-(9–39), which were gifts from Dr. John Eng (VA Medical Center, Bronx, NY). Iodination of GLP-1 was performed as described previously (18).

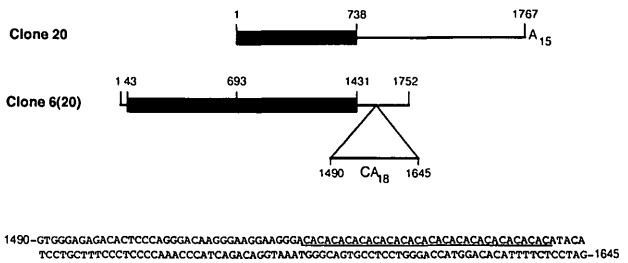

FIG. 1. Schematic representation of the isolated human GLP-1 receptor cDNA clones and sequence of a dinucleotide repeat-containing, probably intronic, segment. Clone hGLPR-20 extends from nucleotide 693 of the coding region down to a polyA tail. Clone hGLPR-6(20) contains the complete coding region and part of the 3′-untranslated region. The sequence of the segment containing the (CA)18 repeat, which is not found in clone hGLPR-20, is also shown. The sequence of both clones is otherwise identical. (■), Coding region; (——), untranslated regions.

cAMP assay. Cells plated in 12-well plates were loaded with 2 mCi tritiated adenine (TRK311, Amersham, Amersham, UK) for 5 h at 37°C. Stimulation with peptides at different concentrations or with 100 mM forskolin was for 8–15 min at 37°C in the presence of 1 mM IBMX. After removal of the medium, cells were lysed with 1 ml of 5% trichloroacetic acid containing 0.1 mM cAMP and 0.1 mM ATP, and the intracellular tritiated cAMP was separated on Sephadex and alumina columns as described previously (26).

RESULTS

Screening of a human λ-gt11 cDNA library was performed with the rat GLP-1 receptor cDNA (18). A single clone with high (83%) nucleotide sequence identity to the rat receptor was isolated. This cDNA clone (hGLPR-20) (Fig. 1) contained only part of the coding region, from nucleotide 689 to the translation termination codon, plus the entire 3′-untranslated region. No other clones for this receptor could be found in this library. A new human islet cDNA library was therefore constructed in the λ-ZAP II vector and screened at high stringency with clone hGLPR-20. One clone (hGLPR-6[20]) (Fig. 1) contained a cDNA coding for a full-length GLP-1 receptor. The deduced amino acid sequence was 90% identical and 96% homologous to the rat receptor (18) (Fig. 2). The cDNA clone hGLPR-6(20) has, in the 3′-untranslated region, a nucleotide sequence not found in clone hGLPR-20 (Fig. 1) and that contains a dinucleotide (CA)18 repeat. This segment may be an intronic sequence that was part of an incompletely spliced mRNA. The boundaries of this sequence indeed conform to the canonical nucleotide sequences bordering the end of introns (26) (Fig. 1). However, the same dinucleotide repeat was found at a similar location in the three previously cloned rat GLP-1 receptor cDNAs (18). Therefore, the presence of this CA repeat in the 3′-untranslated region may be part of, and of functional significance, for the mature receptor mRNA. This region of the receptor mRNA is, however, less

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             .         .         .         .     *   .
  1 MAGAPGPLRLAVLLLGMVGRAGPRPQGATVSLWETVQKWREYRRQCQRSL  50
    ||..|: ||||:|||| |||||||||||||||.||||||||||:|||| |
  1 MAVTPSLLRLALLLLGAVGRAGPRPQGATVSLSETVQKWREYRHQCQRFL  50
             . *▼      .*        . ▼  *    .         .
 51 TEDPPPATDLFCNRTFDEYACWPDGEPGSFVNVSCPWYLPWASSVPQGHV 100
    ||.|  ||:||||||||:|||||||.||||||||||||||||||| ||||
 51 TEAPLLATGLFCNRTFDDYACWPDGPPGSFVNVSCPWYLPWASSVLQGHV 100
       *     .    ▼    .     *   .         .     _____
101 YRFCTAEGLWLQKDNSSLPWRDLSECEESKRGERSWGEEQLLFLYIIYTV 150
    ||||||||:||:||||||||||||||||||.|||...||||| |||||||
101 YRFCTAEGIWLHKDNSSLPWRDLSECEESKQGERNSPEEQLLSLYIIYTV 150
            I          .            II               .
151 AYALSFSALVIASAILLGFRHLHCTRNYIHLNLFASFILRALSVFIKDAA 200
    :|||||||||||||||::||||||||||||||||||||||||||||||||
151 GYALSFSALVIASAILVSFRHLHCTRNYIHLNLFASFILRALSVFIKDAA 200
             .         .              III
201 LKWMYSTAAQQHQWDGLLSYQDSLSCRLVFLLMQYCVAANYYWLLVEGVY 250
    ||||||||||||||||||||||||:|||||||||||||||||||||||||
201 LKWMYSTAAQQHQWDGLLSYQDSLGCRLVFLLMQYCVAANYYWLLVEGVY 250
             .              IV             .         .
251 LYTLLAFSVFSEQWIFRLYVSIGWGVPLLFVVPWGIVKILYEDEGCWTRN 300
    |||||||||||||:||:||:|||||||||||:||||||.|||||||||||
251 LYTLLAFSVFSEQRIFKLYLSIGWGVPLLFVIPWGIVKYLYEDEGCWTRN 300
                  V                         .         .
301 SNMNYWLIIRLPILFAIGVNFLIFVRVICIVVSKLKANLMCKTDIKCRLA 350
    ||||||||||||||||||||||:|:||||||:.|||||||||||||||||
301 SNMNYWLIIRLPILFAIGVNFLVFIRVICIVIAKLKANLMCKTDIKCRLA 350
               VI                .            VII
351 KSTLTLIPLLGTHEVIFAFVMDEHARGTLRFIKLFTELSFTSFQGLMVAI 400
    |||||||||||||||||||||||||||||||:|||||||||||||:|||:
351 KSTLTLIPLLGTHEVIFAFVMDEHARGTLRFVKLFTELSFTSFQGFMVAV 400
             .         .         .         .         .
401 LYCFVNNEVQLEFRKSWERWRLEHLHIQRDSSMKPLKCPTSSLSSGATAG 450
    ||||||||||:||||||||||||:|:||||||||||||||||:|||||.|
401 LYCFVNNEVQMEFRKSWERWRLERLNIQRDSSMKPLKCPTSSVSSGATVG 450
             .
451 SSMYTATCQASCS 463
    ||:|.||||.|||
451 SSVYAATCQNSCS 463
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FIG. 2. Deduced amino acid sequence of the human GLP-1(7–36)amide receptor (upper sequence) and comparison with the rat receptor sequence (lower sequence). The receptors are 90% identical and 95% homologous. Asterisks indicate the positions of the six cysteines, which are perfectly conserved in the extracellular domain of all the members of this G protein–coupled receptor family. Arrowheads denote *N*-glycosylation sites. The position and numbering of the seven transmembrane domains is indicated by horizontal lines above the sequence.

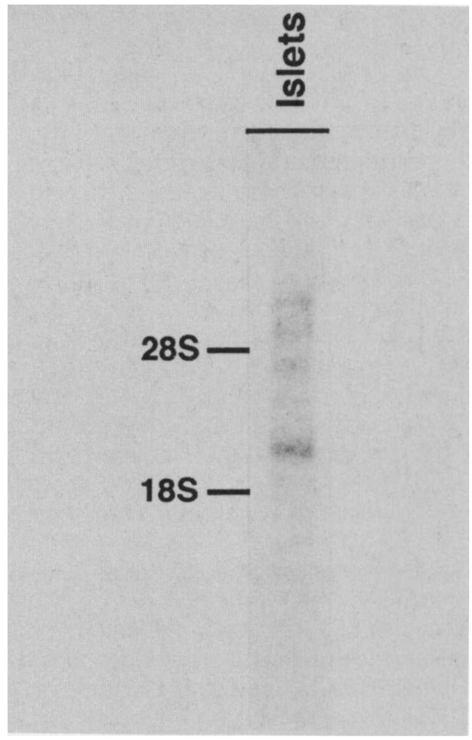

FIG. 3. Northern blot analysis of the GLP-1 receptor transcripts in 0.5 mg polyA RNA from human pancreatic islets. A major 2.6-kb transcript is detected as well as less abundant transcripts of 4.1, 5.0, and 6.0 kb.

conserved between human and rat (62% identity) compared with the nucleotide sequence of the coding region (86% identity). Northern blot analysis of islet polyA RNA with the cloned cDNA revealed the presence of a major 2.6-kb transcript and less abundant transcripts of 4.1, 5.0, and 6.0 kb (Fig. 3). Hybridization of a blot containing 2 μg of polyA RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas did not reveal the presence of any hybridizing bands.

Chinese hamster fibroblasts (CHL cells) transfected with the receptor cDNA displayed high affinity binding sites for [^{125}I]GLP-1(7–36) amide (Fig. 4) with a calculated K_d of 0.5 nM. As for the rat receptor (18), binding of iodinated GLP-1 could not be displaced by GIP, secretin, or VIP (not shown). Binding could be displaced by 70% by oxyntomodulin or by the extended form of GLP-1, GLP-1(1–36)amide, but only when present at a concentration of 1 μM (not shown). Interestingly, however, exendin-4 and exendin-(9–39) displaced radioiodinated GLP-1 binding with IC_{50}s of 0.5 nM and 1.7 nM, respectively. These values were not significantly different from that of GLP-1.

Coupling of the receptor to adenylate cyclase was assessed by measuring cAMP production after exposure of fibroblasts stably expressing the human GLP-1 receptor to increasing concentrations of GLP-1. Figure 5 shows a dose-dependent accumulation of cAMP (11- to 12-fold maximal stimulation over basal, EC_{50} = 93 pM) in response to GLP-1. No GLP-1-induced increase in intracellular cAMP could be detected in cells transfected with

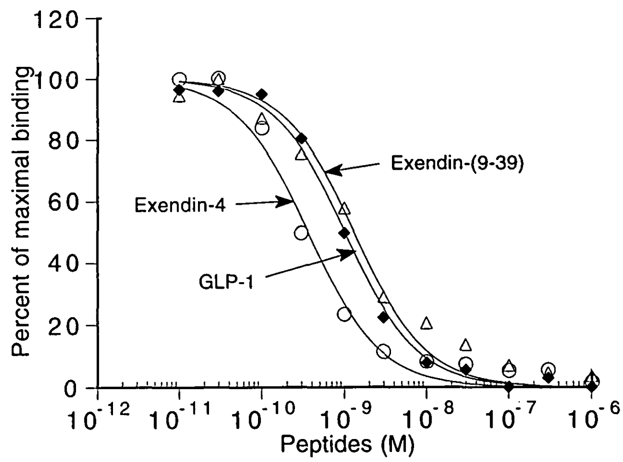

FIG. 4. Displacement of [^{125}I]GLP-1 binding to its receptor by cold peptides. Transfected CHL cells were incubated in the presence of 20 pM radioiodinated GLP-1 and increasing concentrations of GLP-1, exendin-4, or exendin-(9–39) for 15 h at 4°C. GLP-1 displaced radioactive tracer binding with a K_d of 0.5 nM, exendin-4 with a K_d of 0.3 nM, and exendin-(9–39) with a K_d of 1.7 nM. Binding in the absence of competitor peptides and in the presence of 1 μM GLP-1: 291 ± 25 (n = 9) vs. 39 ± 5 cpm (n = 6), respectively.

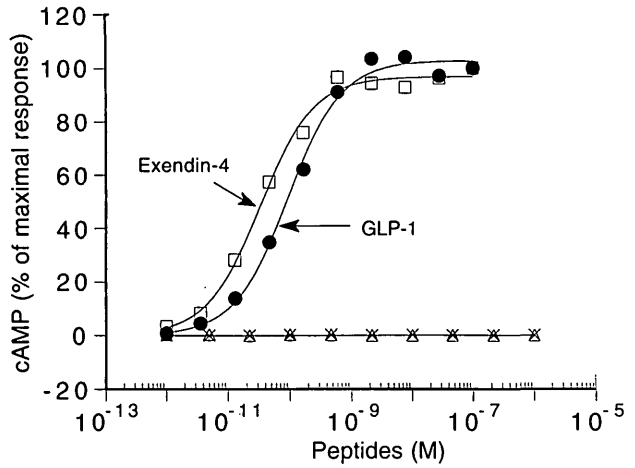

FIG. 5. GLP-1 and exendin-4, but not exendin-(9–39), are agonists of the human GLP-1 receptor. Transfected or nontransfected CHL cells were exposed to increasing concentrations of GLP-1, exendin-4, and exendin-(9–39); and production of cAMP was measured as described in METHODS. In transfected fibroblasts, exendin-4 (□) and GLP-1 (●) induced a dose-dependent increase in cAMP production. Exendin-4 did not however induce cAMP formation in nontransfected fibroblasts (×). Exendin-(9–39) did not induce cAMP formation in receptor-expressing fibroblasts (△). Data are expressed as the percentage of maximal response (100 nM agonist on receptor-transfected cells). Basal cAMP levels (in cpm) in transfected CHL cells and nontransfected cells: 282 ± 132 (n = 6) vs. 637 ± 19 (n = 2), respectively. Maximal cAMP levels in transfected cells, exendin-4 and GLP-1: 3397 ± 220 (n = 2) vs. 3173 ± 97 cpm (n = 2), respectively. Forskolin-induced cAMP levels in transfected and nontransfected cells: 4706 ± 208 (n = 4) vs. 10845 ± 150 (n = 2), respectively.

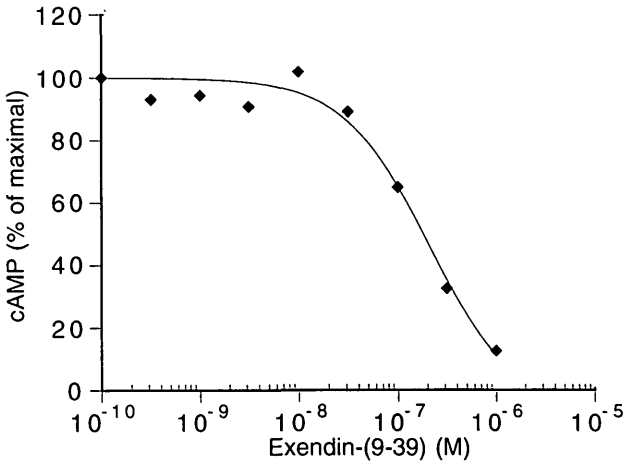

FIG. 6. Exendin-(9–39) is an antagonist of the human GLP-1 receptor. Transfected CHL cells were incubated in the presence of 1 nM GLP-1 and increasing concentrations of exendin-(9–39). The production of cAMP was dose dependently inhibited by exendin-(9–39). Data are expressed as the percentage of maximal response (1 nM GLP-1). Basal cAMP level and the cAMP level in the presence of 1 nM of GLP-1: 205 ± 5 (n = 4) vs. 4210 ± 177 cpm (n = 2), respectively.

the expression vector alone (not shown). Exposure of receptor-transfected fibroblasts, but not of nontransfected CHL cells, to exendin-4 induced the production of cAMP with an EC_{50} of ~33 pM (11- to 12-fold maximal stimulation over basal). Exendin-(9–39) did not induce any detectable production of cAMP in transfected cells. Importantly however, exendin-(9–39) was able to dose dependently block the production of cAMP induced by GLP-1 (Fig. 6). Exendin-(9–39) is therefore an antagonist of the cloned human GLP-1 receptor.

DISCUSSION

Agonists of the GLP-1 receptor might be used as antidiabetic agents (11,13,14). The present characterization of a human pancreatic islet's GLP-1 receptor is therefore of critical importance for the development of new therapeutic agents. Indeed, although highly homologous to the rat receptor (18), the human receptor may display different binding properties for a putative nonpeptide agonist, as has been reported for the human and canine cholecystokinin-B/gastrin receptor (27). Furthermore, we demonstrate that two peptides isolated from the venom of the lizard *Heloderma suspectum*, exendin-4 and exendin-(9–39) (16), which show high sequence similarity to GLP-1, can bind the cloned human receptor with high affinity. In a previous report (28), GLP-1 was shown to displace exendin-4 and exendin-(9–39) from their binding sites on pancreatic acini, suggesting that GLP-1 could bind to a receptor common to these peptides. The molecular nature of the pancreatic acini binding site was not identified. It may have been different from the cloned GLP-1 receptor because in humans (this report) and in rats (18), no GLP-1 receptor mRNA could be detected by Northern blot analysis of total pancreas RNA, although it was abundant in Langerhans islets. Interestingly, whereas exendin-4 acted as an agonist of the receptor, exendin-(9–39) behaved as a perfect antagonist and was able to block GLP-1-induced cAMP formation. Structurally, 7 of 8 amino terminal amino acids are identical between exendin-4 and GLP-1 (28). On the remainder of the molecule, only 9 of 22 amino acids are conserved between exendin-4, exendin-(9–39), and GLP-1. This suggests that some residues in the COOH-terminal end of these peptides may be sufficient for binding but that agonist properties require the presence of the highly conserved 8 amino-terminal residues.

The glucoincretin effect is reduced in diabetic patients, probably because of a defect in binding or signaling by these hormones at the β-cell level (10–12). A lack of or decreased glucoincretin effect by GLP-1 may be a cause in the development of diabetes. The presently described cDNA may therefore also help us understand the regulated expression of this receptor in the development of diabetes, and the identification of a microsatellite in the 3′-untranslated region of the cDNA may, if polymorphic, be used for genetic studies aimed at linking a defect in this gene with development of diabetes.

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The nucleotide sequence of the cloned receptor has

been deposited to GenBank with the accession number U01104.

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