

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 [¹²⁵I]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 glucocretins. The two main glucocretins 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 proglucagon molecule (6). This hormone is a glucocretin 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.

Received for publication 1 July 1993 and accepted in revised form 5 August 1993.

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 *KpnI-PstI* 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 \times SSC (1 \times SSC: 150 mM NaCl, 15 mM Na₃ citrate, pH 7.0), 0.2% SDS, 5 \times Denhardt (1 \times 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 μ g/ml salmon sperm DNA, at 42°C for 18 h. Washings were done 4 times for 20 min in 2 \times 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 μ g 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 *EcoRI*-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 *HindIII-BamHI*-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, Gaithersburg, 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 phenylmethylsulfonyl-fluoride. 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,

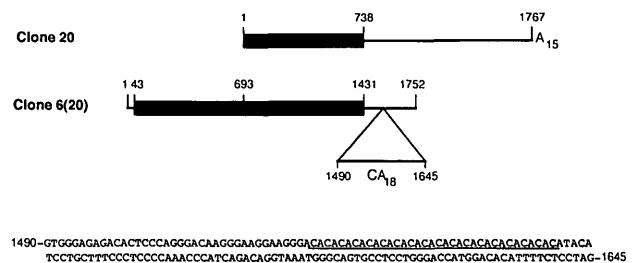


FIG. 1. Schematic representation of the isolated human GLP-1 receptor cDNA clones and sequence of a dinucleotide repeat-containing, probably intronic, segment. Clone hGLPR-20 extends from nucleotide 693 of the coding region down to a polyA tail. Clone hGLPR-6(20) contains the complete coding region and 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.

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).

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

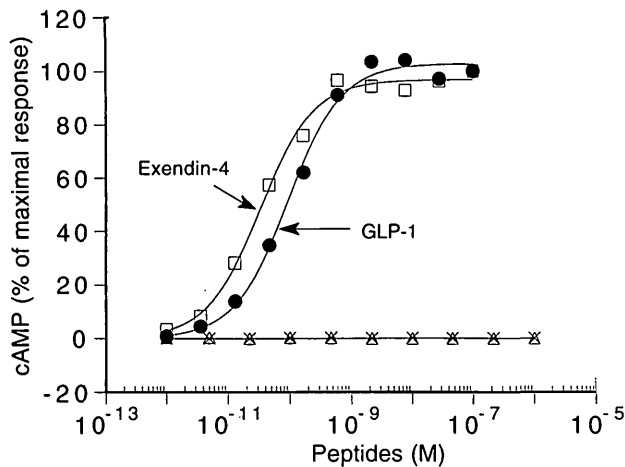


FIG. 5. GLP-1 and exendin-4, but not exendin-9-39, are agonists of the human GLP-1 receptor. Transfected or nontransfected CHL cells were exposed to increasing concentrations of GLP-1, exendin-4, and exendin-9-39; and production of cAMP was measured as described in METHODS. In transfected fibroblasts, exendin-4 (\square) and GLP-1 (\bullet) induced a dose-dependent increase in cAMP production. Exendin-4 did not however induce cAMP formation in nontransfected fibroblasts (\times). Exendin-9-39 did not induce cAMP formation in receptor-expressing fibroblasts (Δ). 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. Forskollin-induced cAMP levels in transfected and nontransfected cells: 4706 ± 208 ($n = 4$) vs. 10845 ± 150 ($n = 2$), 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

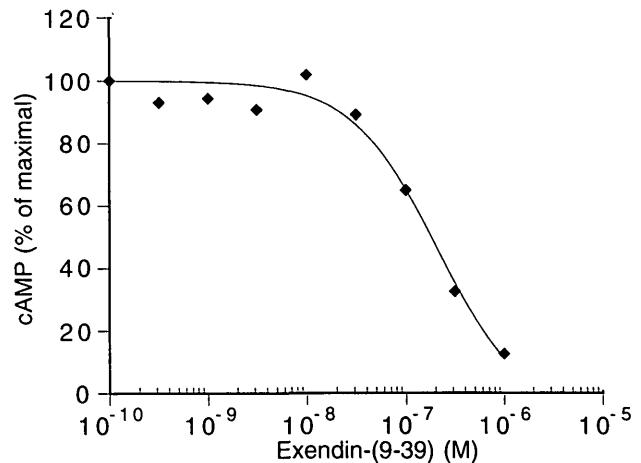


FIG. 6. Exendin-9-39 is an antagonist of the human GLP-1 receptor. Transfected CHL cells were incubated in the presence of 1 nM GLP-1 and increasing concentrations of exendin-9-39. The production of cAMP was dose dependently inhibited by exendin-9-39. Data are expressed as the percentage of maximal response (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.

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 glucocretin 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 glucocretin 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.

ACKNOWLEDGMENTS

This work was supported by Grant 31-30313.90 from the Swiss National Science Fund and Grant 192038 (to B.T.) from the Juvenile Diabetes Foundation International. B.T. is recipient of a Career Development Award from the Swiss National Science Fund.

We thank Dr. John Eng (VA Medical Center, Bronx, New York) for the generous gift of exendin peptides.

The nucleotide sequence of the cloned receptor has

been deposited to GenBank with the accession number U01104.

REFERENCES

1. Ebert R, Creutzfeld W: Gastrointestinal peptides and insulin secretion. *Diabetes Metab Rev* 3:1-16, 1987
2. Unger RH, Eisentraut AM: Entero-insular axis. *Arch Intern Med* 123:261-66, 1969
3. Krarup T: Immunoreactive gastric inhibitory polypeptide. *Endocrine Rev* 9:122-34, 1988
4. Dupre J: *The Endocrine Pancreas*. Samols E, Ed. New York, Raven, 1991, p. 253-28
5. Ørskov C, Bersani M, Johnsen AH, Hojrup P, Holst JJ: Complete sequences of glucagon-like peptide-1 from human and pig small intestine. *J Biol Chem* 264:12826-29, 1989
6. Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L: Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. *J Biol Chem* 261:11880-89, 1986
7. Kreyman B, Ghatei MA, William G, Bloom SR: Glucagon-like peptide-1 (7-36): a physiological incretin in man. *Lancet* II:1300-304, 1987
8. Weir GC, Mojsov S, Hendrick GK, Habener JF: Glucagon-like peptide-1 (7-37) actions on endocrine pancreas. *Diabetes* 38:338-42, 1989
9. Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF: Glucagon-like peptide-1 stimulates insulin gene expression and increases cyclic AMP levels in rat islet cell line. *Proc Natl Acad Sci USA* 84:3434-38, 1987
10. Nauck M, Stöckmann F, Ebert R, Creutzfeld W: Reduced incretin effect in type II (non-insulin-dependent) diabetes. *Diabetologia* 29:46-52, 1986
11. Nauck MA, Heimesaat MM, Ørskov C, Holst JJ, Ebert R, Creutzfeld W: Preserved incretin activity of glucagon-like peptide-1 (7-36)amide but not of synthetic human gastric inhibitory polypeptide in patients with type II diabetes mellitus. *J Clin Invest* 91:301-307, 1993
12. Ørskov C, Jeppesen J, Madsbad S, Holst JJ: Proglucagon products in plasma of non-insulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. *J Clin Invest* 87:415-23, 1991
13. Gutniak M, Ørskov C, Holst JJ, Ahrén B, Efendic S: Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* 326:1316-22, 1992
14. Nathan DM, Schreiber E, Fogel H, Mojsov S, Habener JF: Insulino-tropic action of glucagonlike peptide-1-(7-37) in diabetic and nondiabetic subjects. *Diabetes Care* 15:270-76, 1992
15. Kahn CR, Shechter Y: *The Pharmacological Basis of Therapeutics*. Gilman AG, Rall TW, Nies AS, Taylor P, Eds. New York, Oxford, Pergamon, 1990, p. 1463-94
16. Eng J, Kleinman WA, Singh L, Singh G, Raufman J-P: Isolation and characterization of exendin-4, an exendin-3 analogue, from heloderma suspectum venom: further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J Biol Chem* 267:7402-405, 1992
17. Permutt MA, Koranyi L, Keller K, Lacy PE, Scharp DW, Mueckler M: Cloning and functional expression of a human pancreatic islet glucose transporter cDNA. *Proc Natl Acad Sci USA* 86:8688-92, 1989
18. Thorens B: Expression cloning of the pancreatic β -cell receptor for the gluco-incretin hormone glucagon-like peptide-1. *Proc Natl Acad Sci USA* 89:8641-45, 1992
19. Feinberg AP, Vogelstein B: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 132:6-13, 1983
20. Ricordi C, Lacy PE, Finke EH, Olack B, Scharp DW: An automated method for the isolation of human pancreatic islets. *Diabetes* 37:413, 1988
21. Morel P, Bühler L, Marini M, Deng S, Mage R, Mentha G, Rohner A: Transplantation of human islets of Langerhans. *Schweiz Med Wochenschr* 123:603-608, 1993
22. Gonda TJ, Sheiness DK, Bishop JM: Transcripts from the cellular homologs of retroviral oncogenes: distribution among chicken tissues. *Mol Cell Biol* 2:617-24, 1982
23. Aruffo A, Seed B: Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system. *Proc Natl Acad Sci USA* 84:8573-77, 1987
24. Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain terminating inhibitors. *Proc Natl Acad Sci USA* 74:5463-67, 1977
25. Biggin MD, Gibson TJ, Hong GF: Buffer gradient gels and 35S-label as an aid to rapid DNA sequence determination. *Proc Natl Acad Sci USA* 80:3963-65, 1983
26. Bozou JC, Amar S, Vincent JP, Kitabgi P: Neurotensin-mediated inhibition of cAMP formation in neuroblastoma N1E115 cells: involvement of the inhibitory GTP-binding component of adenylate cyclase. *Mol Pharmacol* 29:489-96, 1986
27. Beinborn M, Lee Y-M, McBride EW, Quinn SM, Kopin AS: A single amino acid of the cholecystokinin-B/gastrin receptor determines specificity for non-peptide antagonists. *Nature (Lond)* 362:348-50, 1993
28. Raufman JP, Singh L, Singh G, Eng J: Truncated glucagon-like peptide-1 interacts with exendin receptors on dispersed acini from guinea pig pancreas. *J Biol Chem* 267:21432-37, 1992