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High Potency Antagonists of the Pancreatic Glucagon-like Peptide-1 Receptor*

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Chahrzad Montrose-Rafizadeh‡§, Huan Yang‡, Buel D. Rodgers‡, Alvie Beday‡, Louella A. Pritchette‡, and John Eng¶‖

From the ‡Laboratory of Clinical Physiology, NIA, National Institutes of Health, Baltimore, Maryland 21224, the ¶Department of Medicine, Veterans Administration Medical Center, Bronx, New York 10468, and the ‖Mt. Sinai School of Medicine, New York, New York 10029

GLP-1-(7–36)-amide and exendin-4-(1–39) are glucagon-like peptide-1 (GLP-1) receptor agonists, whereas exendin-(9–39) is the only known antagonist. To analyze the transition from agonist to antagonist and to identify the amino acid residues involved in ligand activation of the GLP-1 receptor, we used exendin analogs with successive N-terminal truncations. Chinese hamster ovary cells stably transfected with the rat GLP-1 receptor were assayed for changes in intracellular cAMP caused by the test peptides in the absence or presence of half-maximal stimulatory doses of GLP-1. N-terminal truncation of a single amino acid reduced the agonist activity of the exendin peptide, whereas N-terminal truncation of 3–7 amino acids produced antagonists that were 4–10-fold more potent than exendin-(9–39). N-terminal truncation of GLP-1 by 2 amino acids resulted in weak agonist activity, but an 8-amino acid N-terminal truncation inactivated the peptide. Binding studies performed using ^{125}I-labeled GLP-1 confirmed that all bioactive peptides specifically displaced tracer with high potency. In a set of exendin/GLP-1 chimeric peptides, substitution of GLP-1 sequences into exendin-(3–39) produced loss of antagonist activity with conversion to a weak agonist. The results show that receptor binding and activation occur in separate domains of exendin, but they are more closely coupled in GLP-1.

GLP-1-(7–36)-amide (GLP-1)[1] is an incretin hormone that is secreted from the gastrointestinal tract in response to food intake and increases insulin secretion from pancreatic beta cells (1). The physiological action of GLP-1 gained considerable interest following the demonstration that GLP-1 acts on the pancreatic islet beta cells as a potent glucose-dependent insulin secretagogue (2–4). Despite previous structure-function studies of GLP-1 (5–8), no native analog of GLP-1 has been shown to possess potent antagonist activity.

The GLP-1 receptor is a putative seven-transmembrane domain receptor (9) and belongs to the family of G-protein-coupled receptors that includes glucagon-secretin-vasoactive intestinal peptide receptors. GLP-1 binding to the pancreatic beta cell receptor induces an increase in intracellular cAMP levels (10).

Exendin-4-(1–39) (exendin) was originally isolated from Gila monster venom (11) and is a member of the glucagon-secretin-vasoactive intestinal peptide family of peptides. It has 48% amino acid sequence homology to glucagon and 50% homology to human GLP-1. An antagonist, exendin-4-(9–39) (Ex9), was created by the deletion of 8 N-terminal amino acids from exendin (12). Subsequent work by Thorens *et al.* (13) showed that exendin acts directly on the GLP-1 receptor as an agonist, whereas Ex9 acts as an antagonist of the GLP-1 receptor and provided the first high potency antagonist of GLP-1.

The purpose of this study is 2-fold: first, to analyze the transition from agonist to antagonist between exendin and Ex9, and second, to characterize the peptide domains of exendin that confer binding and thus antagonist activity by constructing exendin-(3–39)/GLP-1-(9–36)-amide chimeras and by testing them for the retention of antagonist activity.

MATERIALS AND METHODS

Peptide Synthesis—Peptides were synthesized on a PAL resin solid-phase support using activated Fmoc (*N*-(9-fluorenyl)methoxycarbonyl)-amino acids on a Milligen 9050 automated peptide synthesizer. Cleavage and deprotection of peptides were performed using 90% trifluoroacetic acid, 5% thioanisole, 3% anisole, and 2% ethanedithiol. Crude synthetic peptide mixtures were individually purified by preparative high pressure liquid chromatography. Purified peptides were quantitated by amino acid analysis.

Plasmid Constructs—Full-length GLP-1 receptor cDNA isolated from rat pancreas (gift from Dr. Bernard Thorens, University of Lausanne, Lausanne, Switzerland) was subcloned in pSVbeta (CLONTEC, Palo Alto, CA) downstream of the SV40 promoter after replacing the β-galactosidase gene with a full-length GLP-1 receptor cDNA to obtain pSVGLPR.

Cell Culture and Transfection—CHO cells that overexpress the human insulin receptor (CHO/HIRc cells) were trypsinized and resuspended in Ham's F-12 medium. Cells (10^6 cells in 800 μl) were cotransfected with 10 μg of *Hin*dIII-linearized pSVGLPR plasmid and 1 μg of *Bam*HI-linearized pSVHPH plasmid (conferring hygromycin resistance; American Type Culture Collection, Rockville, MD) by electroporation. Electroporation was performed using a Genepulser (Bio-Rad) in a cuvette with a 0.4-cm gap electrode at 300 mV and 960 microfarads. After a 10-min incubation at room temperature, cells were diluted and plated in multiwell plates and left overnight at 37 °C in a humidified CO_2 incubator. Cells were then treated with 700 μg/ml hygromycin for 10 days, after which single colonies were observed. The clones (CHO/pancGLPR cells) were passaged and allowed to propagate to obtain cells for genomic DNA and RNA preparations. The presence of the GLP-1 receptor in the genomic DNA and the transcripts was observed in six out of eight clones. In addition, the plasmid pSVHPH alone was transfected as a control to obtain CHO/HPH cells.

Western Blot Analysis—Cultures of CHO/HPH or CHO/pancGLPR cells grown to confluence on 60-mm plates were washed three times with Dulbecco's phosphate-buffered saline and frozen in liquid nitrogen. Frozen cells were scraped and solubilized in 50 μl of 2 × Tris/glycine/SDS sample buffer (Novex, San Diego, CA) containing 5% β-mercaptoethanol at room temperature. Cell lysates were cleared by centrifugation for 10 s in aerosol-hydrophobic barrier tips (Para Scientific Co., Fairless Hills, PA) placed in microcentrifuge tubes. Samples were then

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§ To whom correspondence should be addressed: Gerontology Research Center, NIA, NIH, Rm. 2B02, P. O. Box 23, 4940 Eastern Ave., Baltimore, MD 21224.

[1] The abbreviations used are: GLP-1, glucagon-like peptide-1; CHO, Chinese hamster ovary.

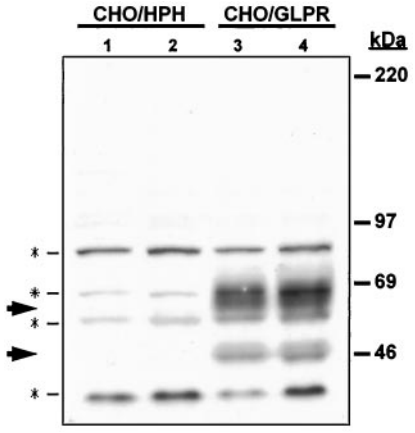

FIG. 1. **Western blot analysis of GLP-1 receptor expression in CHO/pancGLPR and CHO/HPH cells.** CHO cells transfected with the pancreatic GLP-1 receptor (*CHO/GLPR*) or transfected with vector alone (*CHO/HPH*) were solubilized, and GLP-1 receptors were detected after separation by gel electrophoresis and Western blotting with antibody for the amino-terminal region of the GLP-1 receptor. The positions of molecular mass markers are indicated to the right. *Lanes 1* and *3* have equal amounts of cellular extract. *Lanes 2* and *4* have twice the amounts of cellular extract as *lanes 1* and *3*. Nonspecific bands cross-reacting with the antibody are indicated by *asterisks*. Similarly, nonspecific bands have been observed previously in insulinoma cells (14) using the same antibody. The GLP-1 receptor bands are shown with *arrows*.

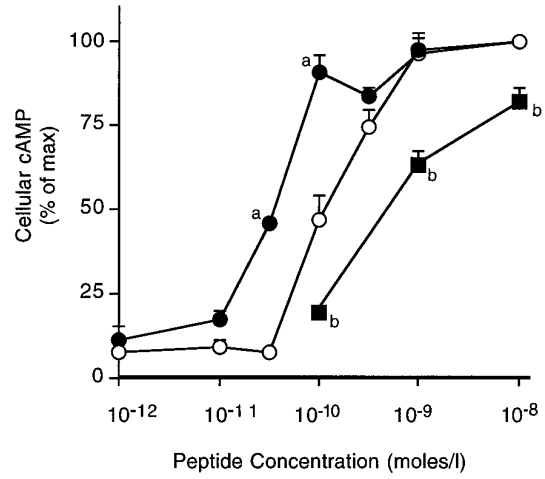

FIG. 2. **Intracellular cAMP levels in the presence of GLP-1 receptor agonists.** Intracellular cAMP levels were measured in CHO/pancGLPR cells exposed for 30 min in the presence of the indicated concentrations of exendin-4-(1–39) (●), GLP-1-(7–36)-amide (○), and exendin-4-(2–39) (■). The data are normalized to maximum values obtained in each experiment in the presence of 10 nM GLP-1. The data points represent the means ± S.E. of three to six experiments performed in duplicate. *a*, $p < 0.01$; *b*, $p < 0.001$ *versus* GLP-1 at the same concentration.

electrophoresed on a 4–12% Tris/glycine gel (Novex) without preheating or boiling and transferred to a 0.2-μm polyvinylidene difluoride membrane (Novex). The membranes were blocked overnight at 4 °C in 5% nonfat dry milk. After washing the membranes in a solution of 20 mM Tris, 137 mM NaCl, and 0.1% Tween 20 (TBST), the membranes were probed with 5 μg/ml anti-N-terminal GLP-1 receptor antibody (a generous gift of Dr. Bernard Thorens) in 1% nonfat dry milk in TBST for 1 h at room temperature followed by horseradish peroxidase-conjugated donkey anti-rabbit antiserum (Amersham Corp.) at a titer of 1:2500 for an additional hour. The membranes were washed three times in TBST for 10 min at room temperature following each antibody incubation. Positive immunoreactions were detected using enhanced chemiluminescence reagents (Amersham Corp.).

Binding of GLP-1 in Intact Cells—Binding studies were performed on cells plated on 12-well dishes and grown to confluence. Cells were washed with serum-free Ham's F-12 medium for 2 h before the experiment. Cells were then washed twice with 0.5 ml of binding buffer containing 120 mM NaCl, 1.2 mM $MgSO_4$, 13 mM sodium acetate, 5 mM KCl, and 10 mM Tris, pH 7.6. Cells were incubated overnight at 4 °C with 0.5 ml of binding buffer containing 2% bovine serum albumin, 500 units/ml aprotinin, 10 mM glucose, 0.03–100 nM GLP-1 or other peptides, and 30,000 cpm ^{125}I-GLP-1 (0.01 nM). At the end of the incubation, the supernatant was discarded, and the cells were washed three times with 0.5 ml of ice-cold Dulbecco's phosphate-buffered saline and incubated at room temperature with 0.5 ml of 0.5 N NaOH and 0.1% SDS for 10 min. Radioactivity in cell lysates was measured in an ICN Apec Series γ-counter. Specific binding was determined as total binding minus the radioactivity associated with cells incubated in the presence of a large excess of unlabeled GLP-1 (0.5 μM).

cAMP Measurements—Transfected CHO cells grown on 12-well plates were washed three times and incubated with 1 ml of Krebs-Ringer phosphate buffer and 0.1% bovine serum albumin for 4 h at 37 °C. Cells were then exposed to Krebs-Ringer phosphate buffer containing 0.1% bovine serum albumin and peptides for 30 min at 37 °C. Cells were washed three times with ice-cold Dulbecco's phosphate-buffered saline and lysed for 5 min with ice-cold 0.6 mM perchloric acid. The pH values of the cell lysates were then adjusted to 7.0 using 5 M K_2CO_3, followed by centrifugation for 5 min at 2000 × *g*. The supernatant was vacuum-dried and solubilized in 500 μl of 500 mM Tris and 4 mM EDTA buffer, pH 7.5. After addition of 50 μl of 0.15 mM Na_2CO_3 and 50 μl of 0.15 mM $ZnSO_4$, followed by incubation for 15 min on ice, the salt precipitate was removed by centrifugation for 5 min at 2000 × *g*, and 50 μl of supernatant was assayed using a [^{3}H]cAMP assay kit (Amersham Corp.). Cellular protein content was measured by the Bradford assay (Bio-Rad) by solubilization of samples and standards in formic acid with γ-globulin as the standard.

RESULTS

The presence of GLP-1 receptor expression in CHO/pancGLPR cells was verified by Western blotting (Fig. 1). Using an antibody against the N-terminal region of the GLP-1 receptor, we obtained specific bands (Fig. 1, *arrowheads*) at 65 and 46 kDa in CHO/pancGLPR cells, but not in CHO/HPH cells. These bands were described previously (14) to correspond to mature and core-glycosylated GLP-1 receptors, respectively. Similar to previously published results (14), this N-terminal antibody also recognizes several other proteins at ~70, 67, and 50 kDa in all CHO cells. Our data suggest that CHO cells transfected with the pancreatic GLP-1 receptor express and process the GLP-1 receptor similar to pancreatic beta cells (14).

We assessed the dose response to GLP-1 and exendin by measuring intracellular cAMP levels in CHO/pancGLPR cells. As shown in Fig. 2, exendin was a more potent agonist than GLP-1. Concentrations of peptide resulting in a half-maximal response were 0.033 ± 0.006 and 0.118 ± 0.02 nM for exendin and GLP-1, respectively ($n = 6$; $p < 0.01$). At the maximum concentration tested (10 nM), both exendin and GLP-1 induced the same maximum rise in cAMP levels (168.8 ± 32.5 and 169.1 ± 31.9 pmol/mg of protein, respectively). Control CHO cells transfected with vector alone (CHO/HPH) did not respond to 10 nM GLP-1 or exendin. The intracellular cAMP content was 1.7 ± 0.4 pmol/mg of protein in the absence of peptides and 2.0 ± 0.4 or 2.0 ± 0.2 pmol/mg of protein in the presence of 10 nM GLP-1 or exendin, respectively. Control experiments confirmed that both agonists acted at a single receptor in CHO/pancGLPR cells since a rise in cAMP levels induced by 10 nM GLP-1 was not further enhanced by 10 nM exendin (data not shown). The results are compared with the response after N-terminal deletion of 1 amino acid from exendin (Ex2). Although agonist activity was maintained with Ex2, the dose-response curve was shifted to the right, and the maximum response was lower than that obtained with 10 nM GLP-1 or full-length exendin.

We studied a series of N-terminal truncated exendin and GLP-1 peptides for agonist and antagonist activities. The series of peptides and the nomenclature are shown in Fig. 3. As shown in Fig. 4*A*, we first examined the effect of progressively truncating a single amino acid from the N terminus of exendin

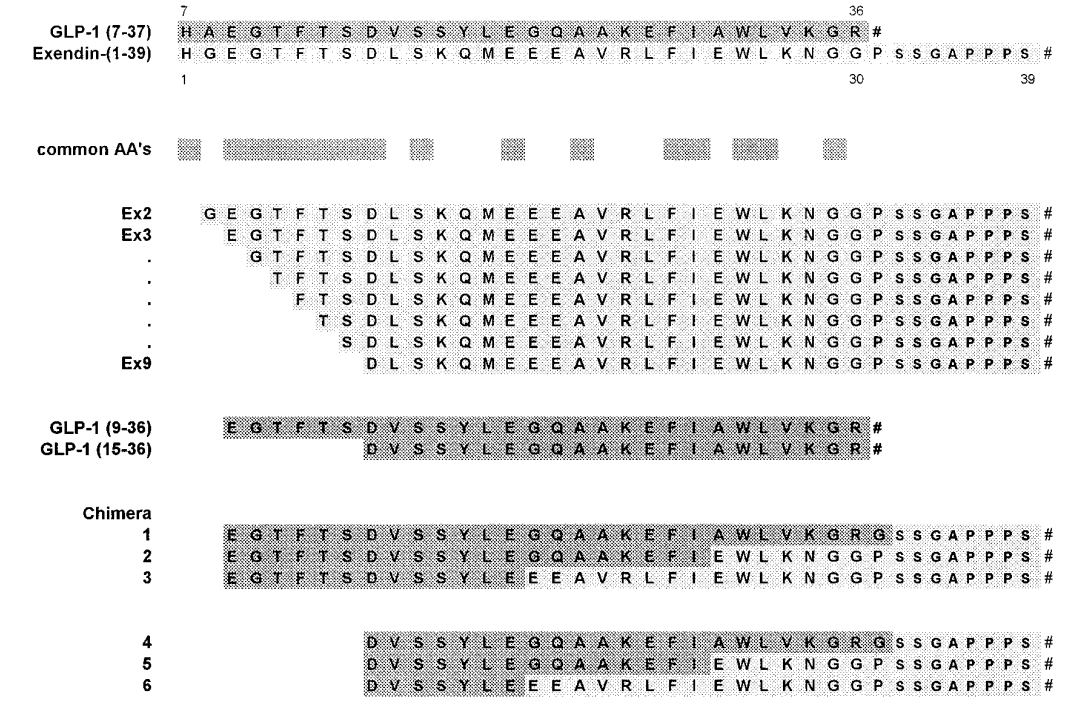

FIG. 3. **Schematic of GLP-1 and exendin sequences and various peptides tested in this study.** The *shaded* areas represent the regions of homology between the GLP-1 and exendin peptides. Position numbers refer to the nomenclature used for GLP-1 (shown in the *darkly shaded* area) and exendin (shown in the *lightly shaded* area). AA's, amino acids.

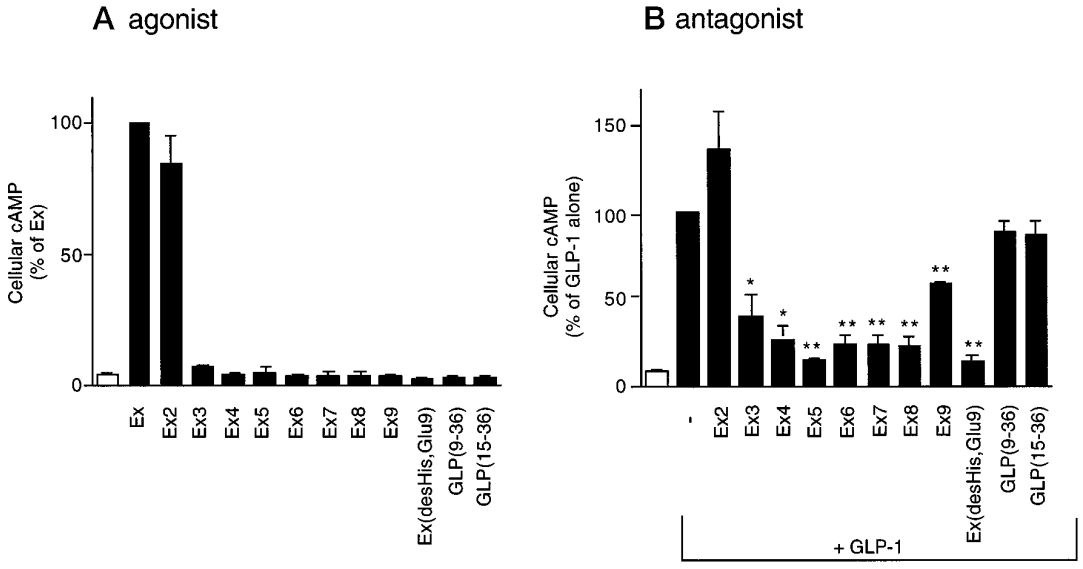

FIG. 4. **Agonist and antagonist activities of N-terminal truncated analogs of exendin and GLP-1.** Intracellular cAMP levels were measured in CHO/pancGLPR cells. *A*, cells were incubated for 30 min in the absence or presence of a 10 nM concentration of various peptides. *Ex*, exendin-4-(1–39); *Ex2–Ex9*, N-terminal truncation of 1–8 amino acids of exendin, respectively; *Ex(desHis,Glu9)*, Ex2 with a substitution of Asp to Glu at position 9; *GLP-(9–36)* and *GLP-(15–36)*, GLP-1-(7–36)-amide with an N-terminal truncation of 2 and 8 amino acids, respectively. *B*, cells were incubated in the absence or presence of 0.1 nM GLP-1. In the presence of GLP-1, the experiments examined the effect of a 10 nM concentration of the indicated peptides. The data points represent the means ± S.E. of three to five experiments performed in duplicate. *, $p < 0.05$; **, $p < 0.01$ *versus* GLP-1 alone.

peptides. As shown above in Fig. 2, Ex2 retained agonist activity. Substitution of the aspartic acid at position 9 with glutamic acid (des-His-[Glu^9]Ex2 (*Ex(desHis,Glu9)*)) completely abolished the agonist activity of Ex2 (Fig. 4*A*). This suggests that this residue is essential to agonist activity as it is in the related glucagon molecule (15). Fig. 4*A* shows that the N-terminal truncation of the exendin peptide by 2–8 amino acids (Ex3–Ex9) as well as the N-terminal truncation of the GLP-1 peptide by 2 or 8 amino acids lead to the loss of agonist activity since these peptides are incapable of elevating intracellular cAMP levels. Therefore, the N-terminal amino acids are important for receptor activation.

Using a half-maximal concentration of GLP-1 (0.1 nM), we assessed the antagonist effects of the various truncated peptides by their ability to lower the GLP-1-induced elevation of cAMP. As shown in Fig. 4*B*, Ex2 (10 nM) had no significant effect. However, Ex3–Ex9 were antagonists since they reduced intracellular cAMP levels induced by GLP-1. Using data obtained from separate experiments, there was a significant inhibition of intracellular cAMP levels by various peptides com-

TABLE I

Binding affinity and antagonist parameters of various GLP-1 analogs

^{125}I-GLP-1 binding to intact CHO/pancGLPR cells was completed with various concentrations of different peptides.

GLP-1 analog	IC_{50}[a]	I/A_{50}[b,c]
GLP-1-(7–36)	1.3 ± 0.5	
GLP-(9–36)	122.4 ± 29.4	
GLP-(15–36)	477.7 ± 139.3	
Exendin-4-(1–39)	0.17 ± 0.04	
Exendin-4-(2–39)	0.21 ± 0.04	
Exendin-4-(3–39)	0.41 ± 0.08	83
Exendin-4-(4–39)	0.3 ± 0.1	41
Exendin-4-(5–39)	0.3 ± 0.1	24
Exendin-4-(6–39)	0.5 ± 0.2	31
Exendin-4-(7–39)	0.4 ± 0.1	41
Exendin-4-(8–39)	0.9 ± 0.2	76
Exendin-4-(9–39)	1.0 ± 0.3	258
Des-His-[Glu^9]Ex2	0.2 ± 0.1	16
Chimera 1	109.8 ± 34.5	
Chimera 2	79.5 ± 11.0	
Chimera 3	6.04 ± 1.07	555
Chimera 4	176.0 ± 40.8	
Chimera 5	272.3 ± 31.4	
Chimera 6	11.4 ± 1.46	674

[a] The concentration that completed 50% of ^{125}I-GLP-1 binding was calculated in three to six separate experiments performed in triplicate. cAMP was measured in CHO/pancGLPR in the presence of GLP-1 and various antagonists.

[b] Values are presented for agonists of analogs with binding affinity that was too low to determine I/A_{50}.

[c] I/A_{50}, the inhibition index is the ratio of the concentration of antagonist to agonist concentration needed to reduce the GLP-1 response to 50% of its response in the absence of an antagonist.

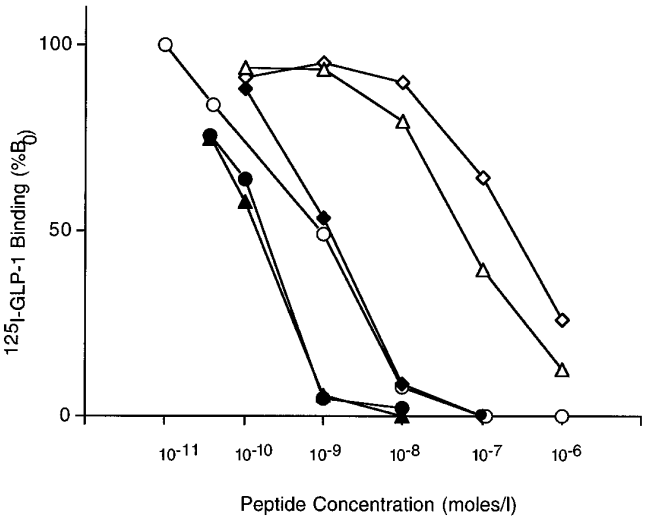

FIG. 5. **Displacement of ^{125}I-GLP-1 binding to CHO/pancGLPR cells with various analogs of exendin and GLP-1.** ^{125}I-GLP-1 binding to intact CHO/pancGLPR cells was competed with various concentrations of different peptides. ○, GLP-1-(7–36)-amide; △, GLP-1-(9–36)-amide; ◇, GLP-1-(15–36)-amide; ●, exendin-4-(1–39); ▲, Ex3; ◆, Ex9. The data points represent the mean of three measurements made in one of several (n = 3–8) representative experiments. The IC_{50} values calculated for various analogs of exendin and GLP-1 are shown in Table I. B_0, maximum binding in the absence of cold peptide.

pared with GLP-1 alone. The substitution of amino acid 9 with glutamic acid in Ex2, des-His-[Glu^9]Ex2 (*Ex(desHis,Glu9)*), converted the peptide to a strong antagonist as it inhibited GLP-1-induced cAMP production by 85.7 ± 3.2% (mean ± S.E., n = 3). Based on these experiments, we also analyzed key truncated GLP-1 peptides for comparison. N-terminal truncation of GLP-1 by 2 or 8 amino acids did not convert the resulting peptides to antagonists since GLP-(9–36) and GLP-(15–36) did not induce a significant inhibition of GLP-1-induced cAMP production (12 ± 7% and 13.4 ± 8.5% decreases, respectively; mean ± S.E., n = 4). Table I compiles information about the antagonist potency (I/A_{50}) of different peptides, showing that Ex3 has ~3-fold more potent antagonist activity than the known Ex9 antagonist. As shown in Table I, there is increased antagonist activity with further N-terminal truncation. The most potent antagonist was obtained after a 4-amino acid truncation of exendin (Ex5).

We performed competition binding studies to assess whether the observed effects among the N-terminal truncated exendin peptides (and lack of activity of various N-terminal truncated GLP-1 peptides) were due to differences in binding affinities for the GLP-1 receptor. As shown in Fig. 5 and Table I, exendin and Ex2–Ex7 displaced ^{125}I-GLP-1 binding with higher affinity than did GLP-1. Ex8 and Ex9 have affinities similar to that of GLP-1. In contrast to the results with exendin peptides, N-terminal truncation of the GLP-1 peptide by 2 and 8 amino acids significantly reduced binding affinity by ~100- and 500-fold, respectively. This suggests that the binding and activation sites are closely associated in the GLP-1 molecule. Control experiments showed no specific binding of ^{125}I-GLP-1 in control CHO/HPH cells transfected with vector alone (data not shown).

To assess which amino acid sequences of exendin are important to confer antagonist properties and high binding affinity, we created chimeras between N-terminal truncated GLP-1 and exendin peptides that contain progressively more C-terminal sequences of exendin substituted into the GLP-1 peptide (chimeras 1–6 in Fig. 3). Using GLP-(9–36) and Ex3 sequences, we created chimeras 1–3. Using GLP-(15–36) and Ex9, we created chimeras 4–6.

As shown in Fig. 6*A*, GLP-(9–36) had 4 orders of magnitude lower agonist potency than the full sequence of GLP-1 or exendin. Substituting increasing amounts of C-terminal exendin sequences (in chimeras 1–3) lowered agonist activity and increased antagonist activity (Fig. 6). As shown in Fig. 6*B*, 100 nM GLP-(9–36) had no effect on cAMP production induced by 0.1 nM GLP-1, but 100 nM chimera 3 inhibited GLP-1-induced cAMP production by 52.0 ± 8.9% (mean ± S.E., n = 3). In one experiment, 1 μM GLP-(9–36) did not inhibit cAMP production induced by 0.1 nM GLP-1. For comparison, 100 nM Ex3 inhibited GLP-1-induced cAMP production by 85.4 ± 4.6% (n = 4).

The difference between chimera 3 and Ex3 suggests that the GLP-1 sequence in chimera 3 contributes to the function of the chimeric peptide. Therefore, we created chimeras 4–6, in which the N-terminal GLP-1 sequences were shortened by 6 amino acids, thus removing the portion of the molecule most homologous between exendin and GLP-1 (see Fig. 3). As shown in Fig. 6, increasing the C-terminal exendin sequence in chimeras 4–6 also increased antagonist activity. The 6 amino acids of GLP-1 retained in chimera 6 continued to affect peptide function. Chimera 6 and Ex9 inhibited the GLP-1-induced cAMP increase by 53.3 ± 3.6% (n = 3) and 70 ± 12% (n = 3), respectively.

As shown in Fig. 7 and Table I, we assessed the ability of the chimeric peptides to displace ^{125}I-GLP-1 binding. The results show that chimeras 1, 2, 4, and 5 have ~2 orders of magnitude lower binding affinity for the GLP-1 receptor than does GLP-1. Increasing the C-terminal exendin sequences in chimeras 3 and 6 increased the binding affinity by an order of magnitude. Chimeras 3 and 6 displaced ^{125}I-GLP-1 binding with only 6–10-fold lower affinity than GLP-1.

DISCUSSION

The model of GLP-1 receptor activation is similar to that of glucagon receptor activation. Two separate steps are important: ligand binding to the receptor, followed by activation of the receptor by the bound ligand (15–18). The occurrence of binding together with activation results in agonist activity.

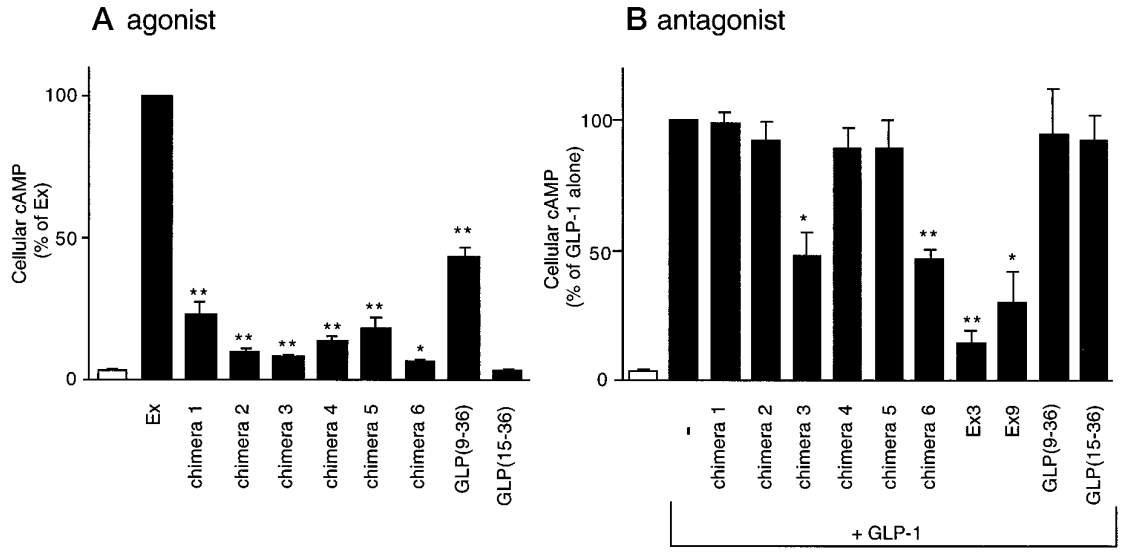

FIG. 6. **Agonist and antagonist activities of various chimeric peptides and various N-terminal truncated analogs of exendin and GLP-1.** Intracellular cAMP levels were measured in CHO/pancGLPR cells. *A*, cells were incubated for 30 min in the absence or presence of 10 nM exendin-4-(1–39) (*Ex*), a 1 μM concentration of various chimeric peptides, or GLP-(9–36) and GLP-(15–36). *, $p < 0.05$; **, $p < 0.01$ *versus* in the absence of peptide (*open bar*). *B*, cells were incubated in the absence or presence of 0.1 nM GLP-1 and in the presence or absence of a 100 nM concentration of the indicated peptide. Ex3 and Ex9 represent the exendin peptide truncated at the N-terminus by 2 and 8 amino acids, respectively. *, $p < 0.05$; **, $p < 0.01$ *versus* GLP-1 alone. The data points represent the means ± S.E. of three to four separate experiments performed in duplicate.

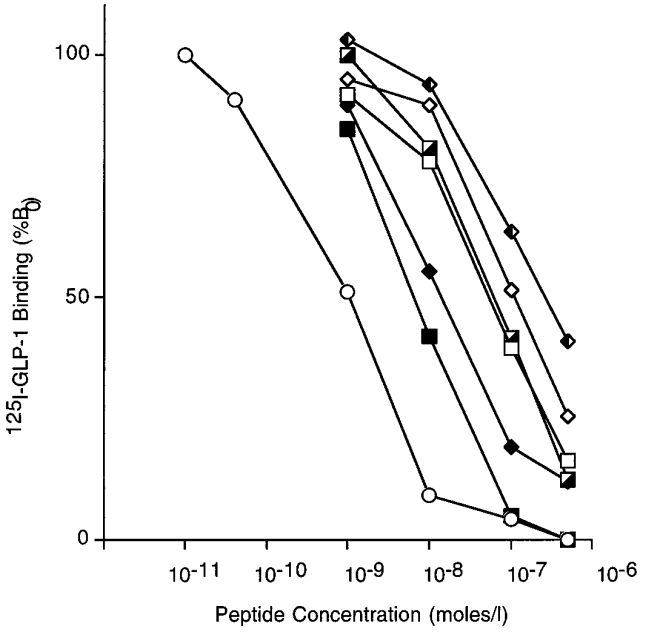

FIG. 7. **Displacement of ^{125}I-GLP-1 binding to CHO/pancGLPR cells with various analogs of exendin and GLP-1.** ^{125}I-GLP-1 binding to intact CHO/pancGLPR cells was competed with various concentrations of the indicated peptides. ○, GLP-1-(7–36)-amide; □, chimera 1; ◪, chimera 2; ■, chimera 3; ◇, chimera 4; , chimera 5; ◆, chimera 6. The data points represent the mean of three measurements made in one of several (n = 3–8) representative experiments. The IC_{50} values calculated for various analogs of exendin and GLP-1 are shown in Table I. B_0, maximum binding in the absence of cold peptide.

However, receptor binding in the absence of receptor activation yields a peptide antagonist. In GLP-1, glucagon, and glucose-independent insulinotropic peptide, the N-terminal region has previously been shown to be important for receptor activation (5, 19, 20). Using exendin, which has a higher affinity for the GLP-1 receptor compared with GLP-1, we have shown that the successive removal of amino acids from the N terminus of exendin disables the ability of the peptides to promote receptor activation. However, these N-terminal truncated peptides continue to bind the receptor with high affinity, thus becoming antagonists. In this study, we have identified several antagonists of the GLP-1 receptor with 3–16-fold higher potency than the previously known antagonist (13), Ex9.

Receptor binding does not predict agonist or antagonist activity since full-length exendin and Ex2 bind with high affinity and are agonists, whereas Ex3–Ex7 bind equally well but are antagonists. However, among agonists or antagonists, receptor binding affinity predicts the potency of action. Full-length exendin (which has a higher binding affinity than GLP-1) was more potent at inducing cAMP production than was GLP-1. Among the antagonists, Ex3–Ex7 have higher binding affinity than Ex8 or Ex9 and also have higher antagonist potency.

The N-terminal domains of exendin and GLP-1 are closely related (see Fig. 3), but manipulation of these closely related sequences had differential effects on receptor binding and activation. Elimination of 2 N-terminal amino acids from exendin (Ex3) yields a peptide that binds with the same affinity as full-length exendin, but antagonizes GLP-1 action. In contrast, truncation of 2 N-terminal amino acids from GLP-1 (GLP-(9–36)) produces a peptide that is a weak agonist with 100-fold lower receptor affinity. Similarly, after truncation of 8 N-terminal amino acids from exendin and GLP-1 peptides, the resultant Ex9 was an antagonist with high affinity, whereas GLP-(15–36) bound with 500-fold lower affinity and became a virtually inactive peptide at the concentrations tested. Our data suggest that similar to glucagon (21), receptor binding and activation are closely associated in the GLP-1 molecule. In contrast, similar amino acid deletions were able to dissociate binding and activation in the exendin molecule. Aspartic acid at position 9 of the exendin peptide seems to be important for receptor activation. Analogous to the glucagon molecule (22), this aspartate substitution in Ex2 did not modify the binding affinity, but converted the peptide to a strong GLP-1 antagonist. Our data suggest that in addition to the GLP-1 molecule, in which sequences in the N terminus (this study) and C terminus (23) are important for receptor binding and activation, sequences within the core portion of exendin (aspartic acid at position 9) are also important for activation.

Dipeptidyl peptidase IV degrades GLP-1 by 2 amino acids,

thereby inactivating the peptide (24–26). In contrast, exendin contains a glycine at position 2 and is predicted to be resistant to the action of dipeptidyl peptidase IV. A previous report (27) showed that GLP-(9–36) is an antagonist of the GLP-1 receptor. However, in that study, high micromolar concentrations of the peptide were used to observe antagonist activity. In our study, we could not detect antagonist activity at 100 nM GLP-(9–36), a concentration that displaced 60% of ^{125}I-GLP-1 binding. In addition, in one experiment, 1 μM GLP-(9–36) was incapable of antagonizing the effect of 0.1 nM GLP-1. It is possible that at higher concentrations and/or higher molar ratios of antagonist to agonist, antagonist activity would be detected. However, we detected only weak agonist activity of GLP-(9–36) at 1 μM. This suggests that GLP-(9–36) may be a partial agonist/antagonist of the GLP-1 receptor.

Using various chimeric peptides, we have identified specific regions in the exendin peptide that are important for receptor binding. Increasing the C-terminal exendin sequence in chimera 1, 2, or 3 and in chimera 4, 5, or 6 increased binding affinity and antagonist activity. Comparisons between chimera 2 *versus* 3 and chimera 5 *versus* 6 suggest that the amino acids within the EEAVRL region of the exendin peptide are important since the addition of this sequence cluster increased the binding affinity by 10–20-fold. In addition, comparisons of chimeras 3 and 6 *versus* Ex3 and Ex9, respectively, suggest that amino acids within the LSKQM region of the exendin peptide can still improve further the binding affinity by 10-fold. Further mutational analysis of individual amino acids in these domains could identify more precisely the sites important for exendin molecule binding to the receptor. The results also have implications for GLP-1 peptide action. Similar comparisons of chimera 2 *versus* 3 and chimera 5 *versus* 6 as well as comparisons of chimeras 3 and 6 *versus* Ex3 and Ex9, respectively, suggest that the amino acids within the GQAAKE and VSSYL regions of GLP-1 could have no effect or a negative regulatory role in binding affinity. These results are in accord with previously published data (5, 6) where substitutions of some amino acids within these regions of GLP-1 with alanine did not have detrimental effects on binding affinity.

In this study, we have identified several potent antagonists of the GLP-1 receptor. Truncating the exendin peptide by 3–6 N-terminal amino acids results in the production of the most potent antagonists of the GLP-1 receptor yet discovered. Structure-function relationships among these various antagonists would allow the creation of models to predict binding sites of the exendin peptide to the receptor. Our data also clearly show that the receptor-binding domain of GLP-1 is distinct from exendin peptide binding. Finally, the use of these potent antagonists of the GLP-1 receptor may allow the discovery of previously unidentified physiological functions of GLP-1 (28).

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