Pergamon

Peptides, Vol. 15, No. 3, pp. 453–456, 1994

0196-9781/94 $6.00 + .00

0196-9781(93)E0032-M

Stable Expression of the Rat GLP-I Receptor in CHO Cells: Activation and Binding Characteristics Utilizing GLP-I(7–36)-Amide, Oxyntomodulin, Exendin-4, and Exendin(9–39)

HANS C. FEHMANN,*[1] JIWEN JIANG,* JOHANNES SCHWEINFURTH,* MICHAEL B. WHEELER,† AUBREY E. BOYD, III† AND BURKHARD GÖKE*

Clinical Research Unit for Gastrointestinal Endocrinology, Department of Medicine, Philipps-University of Marburg, 35033-Marburg, Germany and †Department of Medicine, Division of Endocrinology, Diabetes, Metabolism and Molecular Medicine, New England Medical Center, Tufts University School of Medicine, Boston, MA 02111

Received 9 September 1993

FEHMANN, H. C., J. JIANG, J. SCHWEINFURTH, M. B. WHEELER, A. E. BOYD, III AND B. GÖKE. *Stable expression of the rat GLP-I receptor in CHO cells: Activation and binding characteristics utilizing GLP-I(7–36)-amide, oxyntomodulin, exendin-4, and exendin(9–39).* PEPTIDES **15**(3) 453–456, 1994.—Glucagon-like peptide-I (GLP-I) is a potent insulinotropic peptide that mediates its actions at pancreatic B-cells via specific receptors. In the present study we stably expressed the rat B-cell GLP-I receptor in CHO cells and studied binding characteristics and receptor activation utilizing the naturally occuring receptor agonist GLP-I(7–36)-amide (GLP-I), the proglucagon-derived GLP-I-related peptide oxyntomodulin, the GLP-I receptor agonist exendin-4, and the specific antagonist exendin(9–39). The potencies to displace [^{125}I]GLP-I from the receptor were GLP-I > exendin-4 > exendin(9–39) > oxyntomodulin, and to displace [^{125}I]exendin-4 GLP-I = exendin-4 > exendin(9–39) > oxyntomodulin. cAMP production was stimulated equally by GLP-I and exendin-4. Oxyntomodulin was less potent to stimulate cAMP generation. Exendin(9–39) blocked the stimulatory action of GLP-I and exendin-4 on cAMP production, but not that of oxyntomodulin. This study shows that GLP-I and exendin-4 are potent agonists at the transfected rat B-cell GLP-I receptor whereas oxyntomodulin is only a weak GLP-I receptor agonist. Furthermore, exendin(9–39) is a potent GLP-I receptor antagonist. This peptide is a valuable tool to further study the physiological actions of GLP-I.

Glucagon-like peptide-I (GLP-I) CHO cells Receptor binding Exendin-4 Exendin(9–39) Oxyntomodulin

GLUCAGON-LIKE peptide-I(7–36)-amide/(7–37) (GLP-I) is a potent insulinotropic peptide (10). It is encoded within the proglucagon gene, and the bioactive peptide is mainly expressed in the intestinal L-cell due to a tissue-specific post-translational processing of proglucagon (1,3,21). After a meal, GLP-I is released into the circulation. At pancreatic B-cells GLP-I is a powerful stimulator of glucose-induced insulin secretion and of proinsulin gene expression (6,7,9,16,17). Gulcagon-like peptide-I is presently considered as the most important incretin hormone (2,10). Its action is mediated by receptors expressed by the endocrine pancreatic B-cells (12,18,19,23).

Exendin-4, a peptide containing 39 amino acids recently isolated from Helodermatidae venom (4), was suggested to be an effective agonist at the GLP-I receptor expressed in insulinoma-derived B-cells (13). Furthermore, the *N*-terminally truncated peptide exendin(9–39) was shown to antagonize the action of GLP-I and exendin-4 at B-cells (13). The existence of specific exendin receptors is under discussion, although, in our opinion, it seems more likely that the exendins act via GLP-I receptors (4,13,20).

In the present study we stably expressed the rat GLP-I receptor in the Chinese hamster ovary cell line CHO and studied binding characteristics of GLP-I, exendin-4, exendin(9–39), and oxyntomodulin [glucagon(1–37)] to the recombinant expressed rat GLP-I receptor. Furthermore, we characterized and compared the activation of GLP-I receptor function by GLP-I, exendin-4, and oxyntomodulin and evaluated the effectiveness of exendin(9–39) as an effective peptidergic GLP-I receptor antagonist.

[1] Requests for reprints should be addressed to Dr. Hans C. Fehmann.

METHOD

Glucagon-like peptide-I(7–36)-amide (GLP-I) and GLP-I(7–37) were from Peninsula (Heidelberg, Germany); oxyntomodulin [glucagon(1–37)] was from Sigma (Deisenhofen, Germany). Exendin-4 and exendin(9–39) were synthesized as described previously (13). Glucagon-like peptide-I and exendin-4 were radiolabeled using the iodogen method (iodogen was from Pierce, München, Germany; $Na^{125}I$ was from Amersham, Braunschweig, Germany) and subsequently purified by HPLC. Human serum albumin was from Behring (Marburg, Germany) and bacitracin was from Serva (Heidelberg, Germany). All other chemicals used were from Sigma (Munich, Germany).

Tissue Culture

CHO cells were grown in DMEM medium containing 11 m*M* glucose supplemented with 10% horse serum, 100 U/ml (v/v) penicillin, and 100 μg/ml (v/v) streptomycin; all reagents were obtained from Gibco (Eggenstein, Germany). Cells were split every 3–4 days 1:5.

Production of Stable CHO Cell Lines Expressing the Rat GLP-I Receptor

The isolation and characterization of a cDNA encoding the rat GLP-I receptor, as well as the construction of the expression plasmid, has been described before (25). For the production of stable CHO cell lines expressing the GLP-I receptor, CHO cells (ATCC CCL-61) were transfected with 10 μg of the expression plasmid pGLP-IRNEO using lipofectin™ reagent (BRL, Gaithersburg, MD) according to the manufacturer's instructions. Clones expressing the receptor were generated by G418 (600 μg/ml active, Sigma) selection and pooled. Binding analysis and cAMP measurements were performed to confirm that the pooled clones (CHO-GLP-IR) expressed the receptor and signaled appropriately.

Binding Studies

Binding studies with whole cells were performed as described previously (12). Cells were detached from the plastic dish with cold phosphate-buffered saline containing 136 m*M* NaCl, 2.7 m*M* KCl, 8.1 m*M* Na_2HPO_4, 1.5 m*M* KH_2PO_4, and 1.5 m*M* EDTA, pH 7.3. Cells were centrifuged and carefully resuspended in incubation buffer (2.5 mmol/l Tris/HCl, 120 mmol/l NaCl, 1.2 mmol/l $MgSO_4$, 5 mmol/l KCl, 15 mmol/l CH_3C00Na; pH 7.40) containing 1% human serum albumin, 0.1% bacitracin, and 1 mmol/l EDTA. Approximately 10^6 cells per tube (total volume 300 μl) were incubated for 5 min at 37°C in the presence of unlabeled hormone (1 pmol/l–1 μmol/l) followed by the addition of tracer (25,000 cpm). After 25 min of total incubation time, cells were centrifuged through a mixture of dibutylphthalate/dinonylphthalate (1:1; v/v). Radioactivity in the pellet was counted in a gamma counter. Specific binding was defined as total binding minus unspecific binding (tracer bound in the presence of 1 μmol/l unlabeled hormone).

Determination of cAMP Generation

Cells were detached from the culture dishes with KRB-EDTA and incubated in KRB containing the peptides for 5 min (37°C). In the experiments with exendin(9–39), cells were preincubated with this peptide for 5 min. The reaction was stopped by the addition of 7.5% trichloric acid. Cells were lysed by sonification, and cAMP concentration in cell lysates was measured with a radioimmunoassay kit (Dianova, Hamburg, Germany). Data were calculated as percent of controls (only solvent).

Statistics

Data are expressed as mean ± SEM of six experiments. Statistical analysis was performed by the Student's *t*-test for unpaired data. Statistical significance was set at the 5% level.

RESULTS

[^{125}I]Glucagon-like peptide-I was displaced from CHO GLP-I cells by GLP-I and exendin-4 in a concentration-dependent manner with an EC_{50} of 0.65 nmol/l and 0.8 nmol/l, respectively. Exendin-4(9–39)'s EC_{50} to displace [^{125}I]GLP-I was 2.5 nmol/l (Fig. 1). [^{125}I]Exendin-4 was displaced by GLP-I and exendin-4 with an EC_{50} of 3 nmol/l and by exendin-4(9–39) with an EC_{50} of 6 nmol/l (Fig. 2). No difference was found between GLP-I(7–37) and GLP-I(7–36)-amide to bind to the rat GLP-I receptor (data not shown). Oxyntomodulin displaced both [^{125}I]GLP-I and [^{125}I]exendin-4 only weakly (Figs. 1 and 2): 100 nmol/l oxyntomodulin displaced the tracers by 30% and 40%, respectively. It has been shown previously that glucagon is a weak GLP-I receptor agonist (12).

Glucagon-like peptide-I stimulated cAMP generation concentration dependently, with a maximal effect at 1 nmol/l (500% stimulation). At higher peptide concentrations cAMP production decreased slightly. This phenomenon is most probably be due to a homologous GLP-I receptor desensitization. Exendin-4 stimulated cAMP production with the same potency (500% stimulation), but this peptide showed the tendency to be more efficient then GLP-I, although this difference was not statistically significant. Oxyntomodulin was much less potent (300% stimulation) and efficient to stimulate cAMP generation than GLP-I and exendin-4 (Fig. 3).

Preincubation of CHO GLP-I cells with exendin(9–39) for 5 min significantly blocked the stimulatory action of GLP-I and exendin-4 on cAMP production [10 nmol/l GLP-I: 364%, 10 nmol/l GLP-I + 1 μmol/l exendin(9–39): 208%, $p < 0.05$; 10 nmol/l exendin-4: 353% stimulation, 10 nmol/l exendin-4 + 1 μmol/l exendin(9–39): 155%, $p < 0.01$). Exendin-4(9–39) had

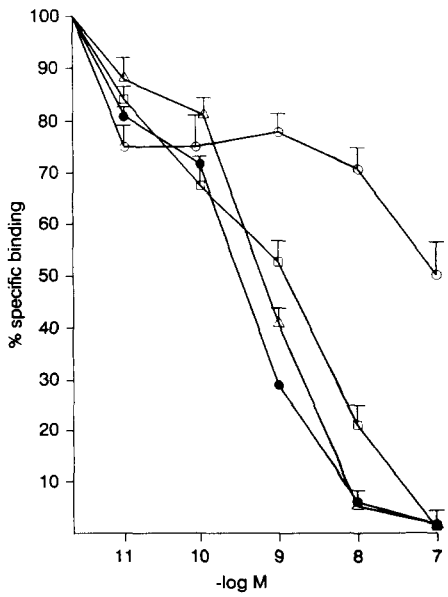

FIG. 1. Displacement of [^{125}I]GLP-I from CHO GLP-I cells expressing the rat GLP-I receptor by GLP-I (●), exendin-4 (△), exendin(9–39) (□), and oxyntomodulin (○).

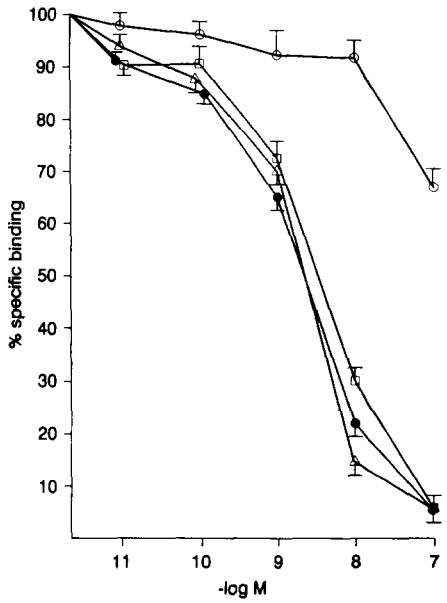

FIG. 2. Displacement of [^{125}I]exendin-4 from CHO GLP-I cells expressing the rat GLP-I receptor by GLP-I (●), exendin-4 (△), exendin(9–39) (□), and oxyntomodulin (○).

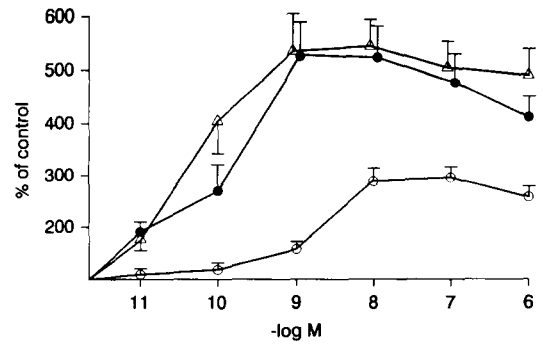

FIG. 3. Effects of GLP-I (●), exendin-4 (△), and oxyntomodulin (○) on cAMP production in CHO GLP-I cells expressing the rat GLP-I receptor. Data are shown as percent of controls (100%).

no significant effect on oxyntomodulin-stimulated cAMP production [10 nmol/l oxyntomodulin: 278%, 10 nmol/l oxyntomodulin + 1 μmol/l exendin(9–39): 212%]. Exendin-4(9–39) had no significant effect on unstimulated cAMP generation (104 ± 8%; Fig. 4).

DISCUSSION

Glucagon-like peptide-I is an important hormonal mediator in the entero-insular axis (10). Its physiological importance is to augment the nutrient-induced postprandial insulin secretion in the presence of elevated glucose levels (14). Furthermore, GLP-I increases insulin production by a direct activation of proinsulin gene transcription (9). In noninsulin-dependent diabetes mellitus (NIDDM), GLP-I is a potent insulin secretagogue in the presence of elevated plasma glucose levels (15,22). Therefore, GLP-I is considered as a new therapeutic agent in noninsulin-dependent diabetes mellitus (NIDDM) (15,22).

Glucagon-like peptide-I belongs to a large family of peptide hormones and all members of this glucagon-secretin-VIP family share at least some degree of sequence homology. On the other hand, these hormones do not interact with the GLP-I receptor. Recently, the 39 amino acid-containing peptide exendin-4, isolated from Helodermatidae venom, was suggested to be an effective agonist at the GLP-I receptor expressed in insulinoma-derived cells (13). Furthermore, exendin(9–39), an *N*-terminally truncated form of exendin-4, was shown to antagonize the effects of GLP-I and exendin-4 at B-cells (13). However, previously the existence of specific exendin receptors has been proposed to explain effects of exendin-4 on the exocrine pancreas where the existence of GLP-I receptors has been excluded (4,11,20,24,25).

Consequently, to further clarify this issue, in the present study we studied binding characteristics and GLP-I receptor activation of GLP-I, exendin-4, oxyntomodulin, and exendin(9–39)-amide to the recombinant rat GLP-I receptor stably expressed in CHO cells. Here we demonstrate that GLP-I, exendin-4, and exendin(9–39) bind with similar affinities to this receptor whereas oxyntomodulin represents only a weak GLP-I receptor agonist. This result argues in favor of the concept that GLP-I, exendin-4, and oxyntomodulin mediate their actions at B-cells via the GLP-I receptor. Furthermore, we have proven that exendin(9–39)-amide represents a powerful rat GLP-I receptor antagonist. This peptide binds to the GLP-I receptor and blocks the actions of GLP-I and exendin-4. In short-term experiments we could not detect any agonistic activity of exendin(9–39) at the rat GLP-I receptor [(13), and this study]. This contrasts to the situation in long-term experiments (24 h) where we previously found a small agonistic effect of exendin(9–39)-amide at B-cells (13). Therefore, exendin(9–39) represents a valuable tool to study the biological significance of GLP-I. The availability of this peptidergic rat GLP-I receptor antagonist now allows determining the contribution of GLP-I to biological-relevant phenomenons as the incretin effect.

Exendin-4 showed the tendency to be more efficient, but equipotent, at the rat GLP-I receptor. This finding agrees with our previous study using insulinoma-derived RINm5F cells (13). Several clinical studies suggested that GLP-I might represent a new therapeutic approach in NIDDM. It is possible to speculate that exendin-4 might be an even more efficient insulinotropic

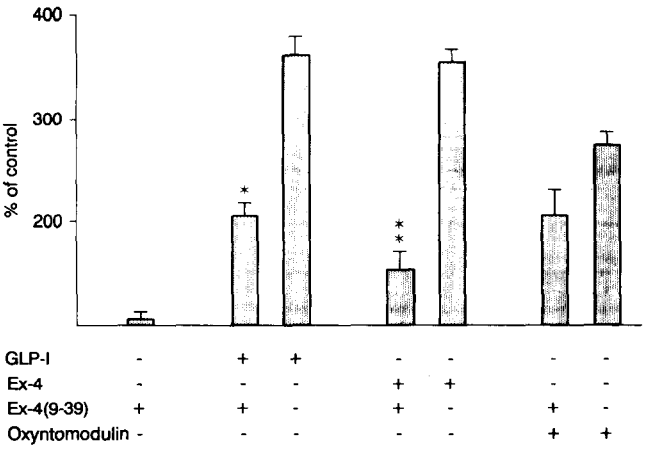

FIG. 4. Effects of exendin-4(9–39) on cAMP production stimulated by GLP-I, exendin-4, and oxyntomodulin in CHO cells expressing the rat GLP-I receptor. Data are shown as percent of controls (100%). GLP-I = GLP-I(7–36)-amide, Ex-4 = exendin-4, Ex-4(9–39) = exendin(9–39). *$p < 0.05$; **$p < 0.01$.

agent in this disease. This idea is supported by a recent study demonstrating exendin-4 as a more potent insulin secretagogue than GLP-I in vivo in dogs (5).

Recently, we have found that exendin-4 binds with a higher affinity to the GLP-I receptor than GLP-I itself expressed in rat insulinoma RINm5F cells (13). In this cell line (CHO) we observed identical binding affinities of both peptides to the overexpressed rat GLP-I receptor. This difference is probably due to different glycosilation of the GLP-I receptor in the different cell lines. Results of previous experiments (R. Just, B. Göke, unpublished results) show that alteration of GLP-I receptor glycosilation drastically impairs GLP-I receptor function.

ACKNOWLEDGEMENTS

We thank Christina Albohn and Harald Schmidt for expert technical assistance. The author's work is supported by the Deutsche Forschungsgemeinschaft (DFG). J. Jiang is a research fellow of the World Health Organization (WHO).

REFERENCES

1. Bell, G. I.; Santerre, R. F.; Mullenbach, G. T. Hamster preproglucagon contains the sequence of glucagon and two related peptides. Nature 302:716–718; 1983.
2. Creutzfeldt, W. The incretin concept today. Diabetologia 16:75–85; 1979.
3. Eissele, R.; Göke, R.; Willemer, S.; et al. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur. J. Clin. Invest. 22:283–291; 1992.
4. Eng, J.; Kleinman, W. A.; Singh, L.; Singh, S.; Raufman, J. P. Isolation and characterization of exendin-4, an exendin-3 analogue, from *Heloderma suspectum* venom. J. Biol. Chem. 267:7402–7405; 1992.
5. Eng, J.; Eng, C. Exendin-3 and -4 are insulin secretagogues. Regul. Pept. 40:142; 1992.
6. Fehmann, H. C.; Göke, B.; Göke, R.; Trautmann, M. E.; Arnold, R. Synergistic stimulatory effect of glucagon-like peptide-I(7–36)amide and glucose-dependent insulin-releasing polypeptide on the endocrine rat pancreas. FEBS Lett. 252:109–112; 1989.
7. Fehmann, H. C.; Göke, R.; Göke, B.; Bächle, R.; Wagner, B.; Arnold, R. Priming effect of glucagon-like peptide-1(7–36)amide, glucose-dependent insulinotropic polypeptide and cholecystokinin-8 at the isolated perfused rat pancreas. Biochim. Biophys. Acta 1091:356–363; 1991.
8. Fehmann, H. C.; Habener, J. F. Homologous desensitization of the insulinotropic glucagon-like peptide-I(7–37) receptor on insulinoma (HIT-T15) cells. Endocrinology 128:2880–2888; 1992.
9. Fehmann, H. C.; Habener, J. F. Insulinotropic hormone glucagon-like peptide-I(7–37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma βTC-1 cells. Endocrinology 130:159–166; 1992.
10. Fehmann, H. C.; Göke, R.; Göke, B. Glucagon-like peptide-I(7–36)amide/(7–37): A new incretin hormone. Mol. Cell. Endocrinol. 85:C39–C44; 1992.
11. Fehmann, H. C.; Göke, B.; Weber, V.; et al. Interaction of glucagon-like peptide-1(7–36)amide and cholecystokinin-8 in the endocrine and exocrine rat pancreas. Pancreas 5:361–365; 1990.
12. Göke, R.; Conlon, J. M. Receptors for glucagon-like peptide-I(7–36)amide on rat insulinoma-derived cells. J. Endocrinol. 116:357–362; 1988.
13. Göke, R.; Fehmann, H. C.; Linn, T.; et al. Exendin-4 is a high potency agonist and truncated exendin(9–39)amide, an antagonist at the glucagon-like peptide 1-(7–36)-amide receptor on insulin-secreting β-cells. J. Biol. Chem. 268:19650–19655; 1993.
14. Göke, R.; Wagner, B.; Fehmann, H. C.; Göke, B. Glucose-dependency of the insulin stimulatory effect of glucagon-like peptide-I(7–36)amide on the rat pancreas. Res. Exp. Med. 193:97–103; 1993.
15. Gutniak, M.; Orskov, C.; Holst, J. J.; Ahren, B.; Efendic, S. Antidiabetogenic affect of glucagon-like peptide-1 (7–36)amide in normal subjects and patients with diabetes. N. Engl. J. Med. 326:1316–1322; 1992.
16. Holst, J. J.; Orskov, C.; Vagn Nielsen, O.; Schwartz, T. W. Truncated glucagon-like peptide 1, an insulin-releasing hormone from the distal gut. FEBS Lett. 21:169–174; 1987.
17. Kreymann, B.; Williams, G.; Ghatei, M. A.; Bloom, S. R. Glucagon-like peptide-1 7–36amide: A physiological incretin in man. Lancet I:1300–1303; 1987.
18. Lankat-Buttgereit, B.; Göke, R.; Stöckmann, B.; Fehmann, H. C.; Göke, B. Detection of the human GLP-1(7–36)amide receptor on insulinoma-derived cell membrane. Digestion 29–33; 1994.
19. Lu, M.; Wheeler, M. B.; Leng, X. H.; Boyd, A. E., III. The role of the free cytosolic calcium level in β-cell signal transduction by gastric inhibitory polypeptide and glucagon-like peptide I(7–37). Endocrinology 132:94–100; 1993.
20. Malhotra, R.; Singh, L.; Eng, J.; Raufmann, J. P. Exendin-4, a new peptide from *Heloderma suspectum* venom, potentiates cholecystokinin-induced amylase release from rat pancreatic acini. Regul. Pept. 41:149–156; 1992.
21. Mojsov, S.; Heinrich, G.; Wilson, I. B.; Ravazzola, M.; Orci, L.; Habener, J. F. Preproglucagon gene expression in pancreas and intestine diversifies at the level of posttranslational processing. J. Biol. Chem. 261:11880–11888; 1986.
22. Nauck, M. A.; Heimesaat, M. M.; Orskov, C.; Holst, J. J.; Ebert, R.; Creutzfeldt, W. Preserved incretin activity of glucagon-like peptide 1(GLP-1) (7–36amide) but not of synthetic human gastric inhibitory polypeptide (GIP) in patients with type 2 diabetes mellitus. J. Clin. Invest. 30:301–307; 1993.
23. Thorens, B. Expression cloning of the pancreatic β cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc. Natl. Acad. Sci. USA 89:8641–8645; 1992.
24. Weber, V.; Fehmann, H. C.; Göke, R.; Göke, B. Effect of proglucagon-derived peptides on amylase release from rat pancreatic acini. Int. J. Pancreatol. 4:325–330; 1990.
25. Wheeler, M. B.; Lu, M.; Dillon, J. S.; Leng, X. H.; Chen, C.; Boyd, A. E., III. Functional expression of the rat glucagon-like peptide-I receptor, evidence for coupling to both adenylyl cyclase and phospholipase-C. Endocrinology 132:57–62; 1993.