Diabetologia (1995) 38: 274–276

Diabetologia

Evidence that glucagon stimulates insulin secretion through its own receptor in rats

K. Kawai[1], C. Yokota[1], S. Ohashi[2], Y. Watanabe[1], K. Yamashita[1]

[1] Department of Internal Medicine, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan
[2] National Institute of Bioscience and Human Technology, Tsukuba, Japan

Summary Since glucagon-like peptide-1 (7–36) amide (7–37) (GLP-1) has been found to be a potent insulinotropic hormone, it has been postulated that glucagon stimulates insulin secretion from islet beta cells through the GLP-1 receptor. We therefore examined the effects of a GLP-1 receptor antagonist, exendin (9–39) amide, on glucagon- or GLP-1-stimulated insulin release from isolated perfused rat pancreas. When infusion of 100 nmol/l exendin (9–39) amide was started 5 min before that of 1 nmol/l glucagon, the stimulation of insulin release by glucagon was similar to that found in the control situation (preinfusion with vehicle alone). By contrast, when 0.3 nmol/l GLP-1 was used in the same experimental setting, exendin (9–39) amide clearly inhibited insulin release. These results indicate that glucagon stimulates insulin release mainly through glucagon receptors but not GLP-1 receptors on islet beta cells. [Diabetologia (1995) 38: 274–276]

Key words Glucagon, insulin secretion, exendin (9–39), GLP-1, pancreas perfusion.

Glucagon stimulates insulin secretion from islet beta cells although its physiologic significance is not yet clear [1]. As a mechanism of this action, it had been generally accepted that insulin secretion is stimulated by glucagon directly through its own receptor on the beta cell, because specific binding sites of ^{125}I-glucagon have been demonstrated in hamster beta-cell tumours [2] and purified beta cells [3]. Since then however, a proglucagon gene product, glucagon-like peptide-1 (7–36) amide/(7–37) (GLP-1), was shown to stimulate insulin secretion more potently [4, 5], and has been accepted as a physiologic insulin secretagogue; a candidate for incretin [6]. Specific receptors for GLP-1 have been detected on rat, mouse, and hamster insulinoma cell lines [7–9], and it is bound by glucagon with an affinity 100 to 1000 times lower than GLP-1 [7, 8]. The insulinotropic activity of glucagon is less potent than that of GLP-1 in the same order [10], leading to the hypothesis that glucagon exhibits its insulinotropic activity through GLP-1 receptors but not glucagon receptors on islet beta cells [11]. Recently, it has been shown that exendin (9–39) amide is a potent receptor antagonist of GLP-1 [12, 13]. In this study, we examined the effects of exendin (9–39) amide on GLP-1 or glucagon-stimulated insulin release from isolated perfused rat pancreas in order to clarify whether or not glucagon stimulates the insulin release through its own receptor.

Received: 2 June 1994 and in revised form: 30 August 1994

Corresponding author: Dr. K. Kawai, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Ibaraki-ken 305, Japan
Abbreviations: GLP-1, Glucagon-like peptide-1; BSA, bovine serum albumin; IRI, immunoreactive insulin.

Materials and methods

Chemicals. Glucagon, GLP-1 (7–36) amide (GLP-1) and exendin (9–39) amide (exendin (9–39)) were synthesized by the stepwise solid-phase method using an automatic synthesizer (model 430A, Applied Biosystem, Foster City, Calif., USA), and then purified by high-performance liquid chromatography (HPLC). The purity of peptides was monitored by analytical reverse-phase HPLC on a column of Nucleosil 5C-18 (4.6 × 150 mm; GL Sciences, Tokyo, Japan) under the isocratic conditions of 0.1 % trifluoroacetic acid and 39 % acetonitril, and proved to be at least 98 % pure [14].

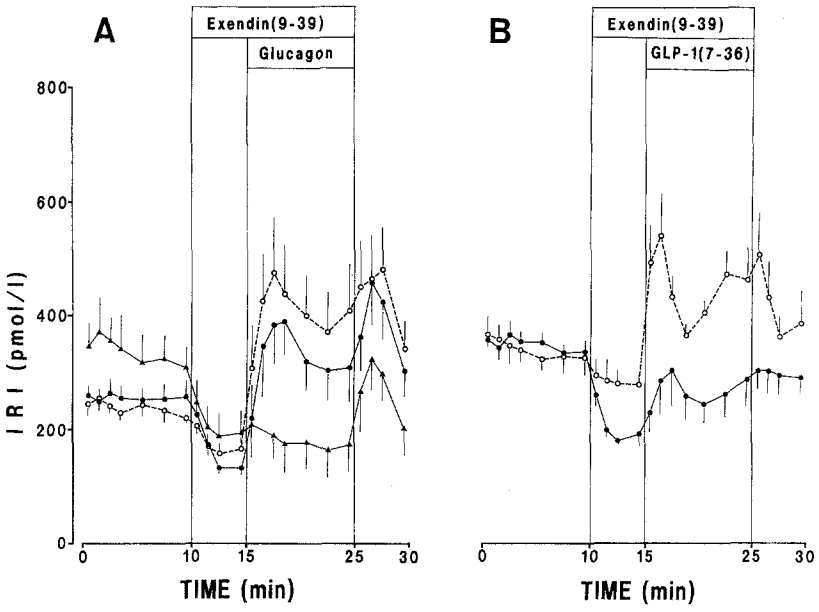

Fig. 1. **A** Effects of 100 nmol/l exendin (9–39) on basal insulin (IRI) release (▲—▲, $n = 5$) and 1 nmol/l glucagon-stimulated insulin release (●—●, $n = 5$) from the isolated perfused rat pancreas. Control study o----o; 1 nmol/l glucagon-stimulated insulin release during the vehicle infusion (0.9 % NaCl containing 0.2 % BSA, $n = 5$).
B Effects of 100 nmol/l exendin (9–39) on 0.3 nmol/l GLP-1-stimulated insulin release (●—●, $n = 6$) from the isolated perfused rat pancreas. Control study o----o ($n = 6$). Results are means ± SEM

Rat pancreas perfusion. The pancreas was isolated in male Wistar rats, weighing 300–350 g, under pentobarbital anaesthesia after an overnight fast. All rats were housed in a windowless, air-controlled room (22 ± 2 °C) under a 12-h light (08.00–20.00) hours/12-h dark cycle, and fed standard rat chow and water ad libitum. Perfusion was carried out according to the method of Grodsky et al. [15]. A Krebs-Ringer bicarbonate buffer solution containing 4 % dextran T-70 (Pharmacia Fine Chemicals, Uppsala, Sweden), 0.2 % bovine serum albumin (BSA), 5 mmol/l each of pyruvate, fumarate, glutamate and arginine, and 5.5 mmol/l glucose was equilibrated with a 95 % O_2-5 % CO_2 mixture at 37 °C, and then continuously gassed throughout the experiment. The pancreas was perfused from the celiac artery at a flow rate of 2 ml/min. After 25 min of equilibration, the effluent perfusate was collected at 1-min intervals by a cannula inserted into the portal vein. There was an initial basal period of 10 min, and then exendin (9–39) dissolved in 0.9 % NaCl solution containing 0.2 % BSA was administered for 15 min through a side-arm syringe at a rate of 0.1 ml/min. Beginning 5 min later, glucagon or GLP-1 dissolved in 0.9 % NaCl containing 0.2 % BSA, 5 mmol/l arginine and 5.5 mmol/l glucose was infused for 10 min through another side-arm syringe at a rate of 0.1 ml/min.

Assay. Immunoreactive insulin (IRI) was determined by RIA according to the method of Herbert et al. [16].

Statistical analysis

The degree of stimulation of insulin release initiated by the peptides was calculated as areas under the curve [14] and compared by Student's *t*-test for unpaired data. $p < 0.05$ was considered significant, and all data are expressed as means ± SEM.

Results

As shown in Figure 1 A, 100 nmol/l exendin (9–39) inhibited insulin release; the percent decrease in ΣΔIRI from the preceding basal level was 40.3 ± 3.6 % ($n = 5$), which is significantly lower than that of the control experiment (infusion of the vehicle; 0.9 % NaCl containing 0.2 % BSA, data not shown); 25.1 ± 5.3 % ($n = 7$, $p < 0.01$). The infusion of 1 nmol/l glucagon for 10 min during 100 nmol/l exendin (9–39) infusion caused an increase in insulin release similar to the increase caused by 1 nmol/l glucagon during the vehicle infusion; the degree of increase in ΣΔIRI from the preceding basal level (mean of 13- and 15-min values) during the exendin (9–39) infusion was 93.1 ± 9.6 % compared to that of the control study.

On the other hand, insulin release caused by 0.3 nmol/l GLP-1 during the 100 nmol/l exendin (9–39) infusion was clearly less than that caused by 0.3 nmol/l GLP-1 during the vehicle infusion (Fig. 1 B); the degree of increase in ΣΔIRI from the preceding basal level (mean of 13- and 15-min values) during the exendin (9–39) infusion was 45.3 ± 3.7 % compared to that of the control study.

Discussion

In the present study, an antagonist of GLP-1, exendin (9–39), clearly inhibited 0.3 nmol/l GLP-1-induced insulin release, but elicited no significant effects on 1 nmol/l glucagon-induced insulin release from the isolated perfused rat pancreas. These results indicate that glucagon stimulates insulin release mainly through glucagon receptors but not GLP-1 receptors on islet beta cells. In other words, if there were no glucagon receptors on beta cells [17] and glucagon stimulates insulin release through GLP-1 receptors, the insulin release caused by 1 nmol/l glucagon should be inhibited more clearly than that by 0.3 nmol/l GLP-1. A similar result has been reported in an in

vivo study using fasted dogs [18]. In our direct comparison of the insulinotropic activity of glucagon superfamily-peptides, the half-maximum effective dose (ED_{50}) for glucagon was 25 times greater than that for GLP-1 (2.7 nmol/l and 50 pmol/l, respectively) [19], while the binding of ^{125}I-GLP-1 to GLP-1 receptor-transfected COS cells was inhibited to only 50 % by 1 μmol/l glucagon [20]. These results also support the conclusion of the present study.

In this study, 100 nmol/l exendin (9–39) itself inhibited insulin release. This inhibition has been found to be dose dependent (unpublished observation). This suggests that beta-cell GLP-1 receptors in this study may in some way be activated leading to elevated basal insulin release which could then be counteracted by the antagonist. Alternatively, exendin (9–39) might modulate insulin release through other direct or indirect mechanisms. It will be interesting to study this in the future.

Acknowledgements. The authors wish to thank Ms. K. Sasaki for her excellent technical assistance. This study was supported by a grant from the University of Tsukuba Research Project.

References

1. Samols E, Marris G, Marks V (1965) Promotion of insulin secretion by glucagon. Lancet 2: 415–417
2. Goldfine ID, Roth J, Birnbaumer L (1972) Glucagon receptors in β-cells: binding of ^{125}I-glucagon and activation of adenylate cyclase. J Biol Chem 247: 1211–1218
3. Van Schravendijk CFH, Foriers A, Hooghe-Peters EL et al. (1985) Pancreatic hormone receptors on islet cells. Endocrinology 117: 841–848
4. Holst JJ, Ørskov C, Nielsen OV et al. (1987) Truncated glucagon-like peptide-I: an insulin-releasing hormone from the distal gut. FEBS Lett 211: 169–174
5. Mojsov S, Weir GC, Habener JF (1987) Insulinotropin: glucagon-like peptide I (7–37) actions on endocrine pancreas. J Clin Invest 79: 616–619
6. Creutzfeldt W, Nauck M (1992) Gut hormones and diabetes mellitus. Diab Metab Rev 8: 149–177
7. Ørskov C, Nielsen JH (1988) Truncated glucagon-like peptide-1 (proglucagon 78–107 amide), an intestinal insulin-releasing peptide, has specific receptors on rat insulinoma cells (RIN 5 AH). FEBS Lett 229: 175–179
8. Fehmann HC, Habener JF (1991) Functional receptors for the insulinotropic hormone glucagon-like peptide-1 (7–37) on a somatostatin secreting cell line. FEBS Lett 279: 335–340
9. Fehmann HC, Habener JF (1991) Homologous desensitization of the insulinotropic glucagon-like peptide-I (7–37) receptor on insulinoma (HIT-T15) cells. Endocrinology 128: 2880–2888
10. Weir GC, Mojsov S, Hendrick GK et al (1989) Glucagon-like peptide I (7–37) actions on endocrine pancreas. Diabetes 38: 338–342
11. Fehmann H-C, Göke R, Göke B (1992) Glucagon-like peptide-1 (7–37)/(7–36) amide is a new incretin. Mole Cell Endocrinol 85: C39–C44
12. Raufman J-P, Singh L, Singh G et al. (1992) Truncated glucagon-like peptide-1 interacts with exendin receptors on dispersed acini from guinea pig pancreas. J Biol Chem 267: 21 432–21 437
13. Göke R, Fehmann H-C, Linn T et al. (1993) 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 of insulin-secreting β-cells. J Biol Chem 268: 19 650–19 655
14. Kawai K, Suzuki S, Ohashi S et al. (1989) Comparison of the effects of glucagon-like peptide-1 (1–37) and -(7–37) and glucagon on islet hormone release from isolated perfused canine pancreas. Endocrinology 124: 1768–1773
15. Grodsky GM, Batts AA, Bennett LL et al. (1963) Effects of carbohydrates on secretion of insulin from isolated rat pancreas. Am J Physiol 205: 638–644
16. Herbert V, Lau KS, Gottlieb CW et al. (1965) Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25: 1375–1384
17. Kofod H, Adelhorst K, Christensen LB et al. (1993) Beta-cells do not have glucagon receptors but GLP-1 receptors. Digestion 54: 346–347
18. Eng J, Gutterman V, Nicolis C (1993) Exendin (9–39) amide inhibits the insulin secretagogue activity of exending 4 and GLP-1 but enhanced that of glucagon in dogs. Digestion 54: 354
19. Suzuki S, Kawai K, Ohashi S et al. (1992) Comparison of the insulinotropic activity of glucagon-superfamily peptides in rat pancreas perfusion. Horm Metab Res 24: 458–461
20. Thorens B (1992) 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