Glucagonostatic and Insulinotropic Action of Glucagonlike Peptide I-(7–36)-Amide

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We examined the effect of glucagonlike peptides (GLPs), which are cleaved from preproglucagon in the enteroglucagon cells, on rat endocrine pancreas with the isolated perfused system. GLP-I-(7–36)-amide, a truncated form of full-sequence GLP-I-(1–37), showed a potent inhibitory effect on glucagon secretion. This inhibitory effect of GLP-I-(7–36)-amide was demonstrated at concentrations of 0.25, 2.5, and 25 nM in 11.2 and 2.8 mM glucose. In contrast, insulin release was significantly stimulated by GLP-I-(7–36)-amide at its concentration from 0.025 to 25 nM in a high glucose concentration, whereas in a low glucose concentration, the stimulation was seen only at the highest concentration (25 nM). Neither GLP-I-(1–37) nor GLP-II showed any effect on glucagon and insulin release. Although several gastrointestinal hormones have been nominated as incretins, none of them may suppress the glucagon secretion. A truncated form of GLP-I, GLP-I-(7–36)-amide thus seems to be a unique incretin that exerts glucagonostatic action. *Diabetes* 38:902–905, 1989

The structure of mammalian preproglucagon has been deduced from nucleotide sequencing of cDNA derived from mRNA from several species (1–5). Preproglucagon contains two other glucagonlike peptides (GLPs), GLP-I and GLP-II, within the COOH-terminal half of the molecule. The amino acid sequence of GLP-I-(37 amino acid residues) is completely conserved among the five mammalian species that have been studied (5). This remarkably high degree of conservation suggests that GLP-I may have a significant biological function. But it has been already reported that there was a weak insulinotropic effect of synthetic GLP-I-(1–36)-amide but not of synthetic GLP-II-(1–34) on isolated rat islets (6), and there was no effect of synthetic GLP-I-(1–37) on insulin secretion in the isolated perfused rat pancreas (7). GLP-I-(7–37) (7) and GLP-I-(7–36)-amide (8,9) potently stimulate insulin secretion in vitro and in vivo. These truncated forms of GLP-I are processed from preproglucagon in the intestine (10) and secreted into the circulation (9). GLP-I-(7–37) potently increased cAMP levels, insulin mRNA transcripts, and insulin release in cultured rat insulinoma cells (11). Thus, the biologically active forms of GLP-I could be reasonably postulated to be the new candidates for incretins. Although gastric inhibitory polypeptide (GIP), the most attractive incretin candidate so far, was also reported to exert a stimulatory effect on glucagon secretion (12,13), the effect of GLPs on glucagon secretion has not been studied.

In this study, we examined the effect of GLP-I-(7–36)-amide, full-sequence GLP-I-(1–37), and GLP-II on glucagon secretion in comparison with the effect on insulin secretion with the perfused rat pancreas.

Glucagon	1 ng/L = 1 pg/ml	Insulin	1 pM = 0.139 μU/ml
Glucose	1 mM = 18 mg/dl		

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RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats weighing 180–250 g were fasted overnight and anesthetized with 25 mg/kg i.p. pentobarbital sodium (Somnopentyl, Pitman-Moore, Washington Crossing, NJ). The pancreas was isolated from the vascular system except for its connections with the duodenum and was perfused through the celiac artery by means of a cannula inserted into the abdominal aorta. All other aortic branches were ligated. The effluent from the pancreas was collected through a second cannula placed in the portal vein. The perfusion medium was Krebs-Ringer bicarbonate buffer containing 4.5% dextran (Dextran T 70, Pharmacia, Uppsala, Sweden), 0.1% bovine serum albumin (fraction V, Daiichi, Tokyo), and 2.8 or 11.2 mM of glucose and was gassed

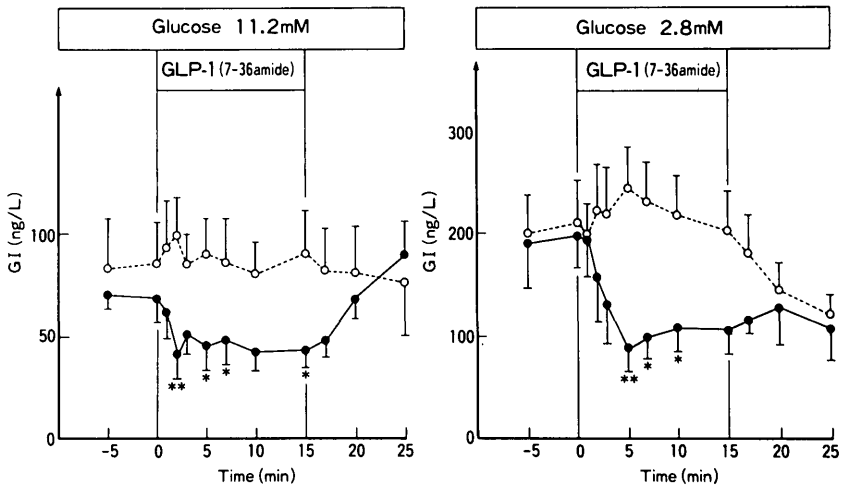

FIG. 1. Effect of 25 nM glucagonlike peptide I (GLP-1) (7–36)-amide (●) on glucagon immunoreactivity (GI) secretion from perfused rat pancreas in 11.2 or 2.8 mM glucose. Control experiments (○) were perfused without peptide. Values are means ± SE; $n = 6$. *$P < .05$, **$P < .01$, vs. control.

constantly with 95% O_2/5% CO_2 to achieve pH 7.4. The preparation was perfused for 20 min with control medium and for 15 min with a medium containing various concentrations (0.025–25 nM) of synthetic GLP-I-(7–36)-amide (lot no. 010900) and 25 nM of GLP-I-(1–37) (lot no. 009795) and GLP-II-(1–34) (lot no. 008674); these peptides were obtained from Peninsula (Belmont, CA). After that, the perfusion with control medium was resumed. A multichannel roller pump (Minipuls 2, Gilson, Villiers-le-Bel, France) was used to maintain a constant perfusion rate of 2 ml/min. All experiments were carried out at 37°C and were completed within 50 min. The viability of the perfused pancreas was confirmed by 20 mM arginine stimulation after the experiment. The portal venous effluent was collected at 1-min intervals and stored at −20°C in tubes containing 500 KIU/ml aprotinin (Antagosan, Hoechst, Frankfurt, FRG) until the analyses for glucagon and insulin.

Glucagon immunoreactivity (GI) was measured by radioimmunoassay (RIA) as previously described (14) with a minor modification, i.e., use of AGS 18, an antiserum specific for the COOH-terminal of the glucagon molecule. The characteristics of AGS 18 have been described elsewhere (15,16). Immunoreactive insulin (IRI) was measured by the Phadeseph insulin RIA kit (Pharmacia) with rat insulin (Novo, Bagsvaerd, Denmark) as standard.

The results are expressed as means ± SE of five or six experiments. ΣΔIRI and ΣΔGI values were calculated as the area under or over the response curves from basal values at time 0 for a 15-min test period with various peptides, respectively. Statistical analysis of the results was performed by analysis of variance with F tests. When $P < .05$ by F test, the differences between groups were examined with the Scheffé multiple-comparisons test or the Wilcoxon-Mann-Whitney test.

RESULTS

The basal level of GI in the 11.2 mM glucose was lower than that in 2.8 mM glucose (83 ± 24 vs. 198 ± 38 ng/L, both in control experiments, $P < .05$; Fig. 1). Twenty-five nanomoles of synthetic GLP-I-(7–36)-amide significantly suppressed glucagon secretion from 68.5 ± 9.0 to 41.5 ± 11.5 ng/L in 11.2 mM glucose ($P < .01$ vs. control) and from 196.0 ± 32.5 to 87.0 ± 23.5 ng/L in 2.8 mM glucose ($P < .01$ vs. control) within 5 min after beginning the peptide perfusion. A significant inhibitory effect of GLP-I-(7–36)-amide on glucagon secretion was evidenced at a peptide concentration >0.25 nM in both glucose concentrations ($P < .05$ vs. control; Table 1). The mean values of the decremental area of glucagon secretion after GLP-I-(7–36)-amide administration appeared to be maximal at 2.5 nM in both glucose concentrations (Table 1). In the low glucose concentration, maximal glucagon suppression occurred at 2.5 nM by the statistical analyses with multiple comparisons ($P < .05$ vs. 0.25 or 25 nM) but did not occur statistically in the high glucose concentration.

GLP-I-(7–36)-amide stimulated insulin release biphasically at both glucose concentrations irrespective of the basal IRI level (Fig. 2). The responses of insulin release were much

TABLE 1
Dose-response effect of glucagonlike peptide I (GLP-I)-(7–36)-amide on glucagon and insulin secretion in glucose

		GLP-I-(7–36)-amide (nM)			
	Control	0.025	0.25	2.5	25
Glucose, 2.8 mM					
ΣΔGI (ng · min · L^{-1})	108 ± 219	336 ± 183	−500 ± 171*	−2986 ± 321†	−1332 ± 305†
ΣΔIRI (pM/min^{-1})	−17 ± 44	22 ± 26	19 ± 29	119 ± 51	431 ± 158*
Glucose, 11.2 mM					
ΣΔGI (ng · min · L^{-1})	45 ± 130	−2 ± 136	−230 ± 63*	−719 ± 232*	−398 ± 52*
ΣΔIRI (pM/min^{-1})	−210 ± 179	1040 ± 166†	2613 ± 258†	2082 ± 222†	3019 ± 374†

Values are means ± SE of incremental areas between basal levels and response curves in 5 or 6 experiments. ΣΔGI, glucagon immunoreactivity; ΣΔIRI, insulin immunoreactivity. ΣΔGI and ΣΔIRI values are calculated as the area under or over the response curves from basal values at time 0 for 15-min test period with various peptides, respectively.
*$P < .05$, †$P < .01$, vs. control experiments.

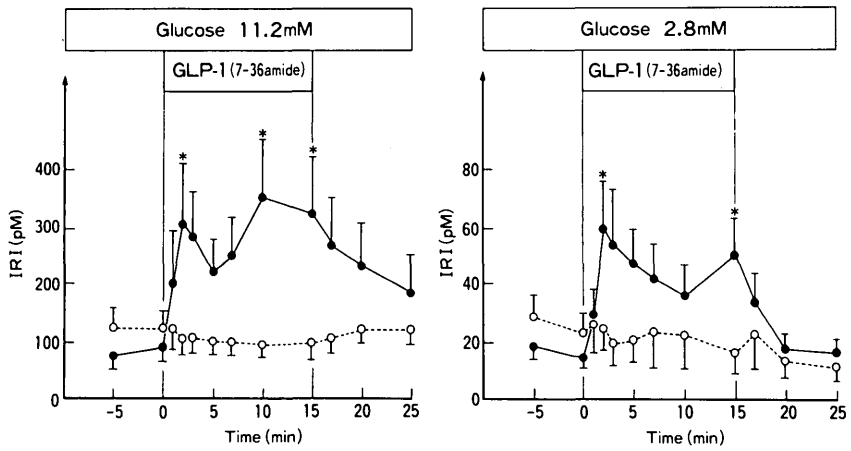

FIG. 2. Effect of 25 nM glucagonlike peptide I (GLP-1) (7–36)-amide (●) on immunoreactive insulin (IRI) secretion from perfused rat pancreas in 11.2 or 2.8 mM glucose. Control experiments (○) were perfused without peptide. Values are means ± SE. *n* = 6. **P* < .05 vs. control.

greater in the high glucose concentration than in the low glucose concentration (Table 1). At the low glucose concentration, significant insulin release was only observed with 25 nM of GLP-I-(7–36)-amide, whereas at 11.2 mM glucose, an effect on insulin secretion occurred when 0.025 nM of the peptide was administered. In addition, at this glucose concentration, the magnitude of the insulin response was not different when concentrations of GLP-I-(7–36)-amide were increased >0.25 nM.

Synthetic full-sequence GLP-I-(1–37) did not affect glucagon secretion (Fig. 3) or insulin release (Fig. 4), regardless of the glucose concentration.

GLP-II showed no effect on glucagon release and no effect on insulin release (Figs. 3 and 4).

At the end of control-buffer perfusion, insulin responded from 149 ± 23 to 761 ± 129 pM in 11.2 mM glucose and from 15 ± 3 to 36 ± 3 pM in 2.8 mM glucose; glucagon responded from 76 ± 26 to 854 ± 396 ng/L in 11.2 mM glucose and from 162 ± 33 to 1386 ± 478 ng/L in 2.8 mM glucose by 20 mM arginine stimulation.

DISCUSSION

This study demonstrates that GLP-I-(7–36)-amide not only stimulates insulin release but also clearly suppresses glucagon secretion in high and low glucose concentrations. Full-sequence GLP-I-(1–37) and GLP-II-(1–34) had no effect on insulin secretion as previously reported (6,7). We confirmed the viability of the islets in this perfusion system by normal responsiveness to 20 mM arginine stimulation in control experiments. Therefore, the suppression of GI level observed in this study is unlikely to be due to a failure of the secretory function of the α-cells.

Several gastrointestinal hormones have been nominated as incretins, which exert a glucose-dependent insulinotropic action (17,18), although none of the incretins may suppress glucagon secretion. GIP, the most convincing candidate for an incretin so far, reportedly stimulates glucagon release from the perfused rat pancreas in low glucose concentrations (13). The inhibition of glucagon secretion by GLP-I-(7–36)-amide seems to be unique in this aspect, which suggests the importance of GLP-I in the regulation of pancreatic glucagon and insulin secretion. Furthermore, in high glucose concentrations, GLP-I-(7–36)-amide stimulated insulin release at a relatively low concentration (0.025 nM) in this study. GIP has been reported to stimulate insulin release scarcely at 0.2 nM concentration in a similar glucose concentration (8.9 mM; 13). Thus, GLP-I-(7–36)-amide possibly has a more potent insulinotropic activity than GIP. The inhibitory effect of GLP-I-(7–36)-amide on glucagon secretion may be directly exerted, although it could be mediated by endogenous insulin (19) and/or somatostatin (20,21). However, our data that glucagon secretion was also suppressed

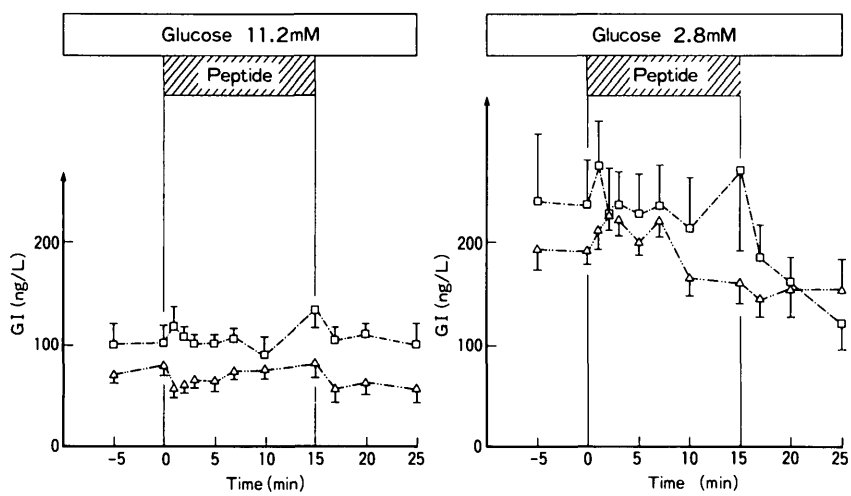

FIG. 3. Effect of 25 nM glucagonlike peptide I (GLP-I)-(1–37) (□) or 25 nM GLP-II-(1–34) (△) on glucagon immunoreactivity (GI) secretion from perfused rat pancreas in 11.2 or 2.8 mM glucose. *Peptide* indicates GLP-I-(1–37) or GLP-II-(1–34). Values are means ± SE; *n* = 6.

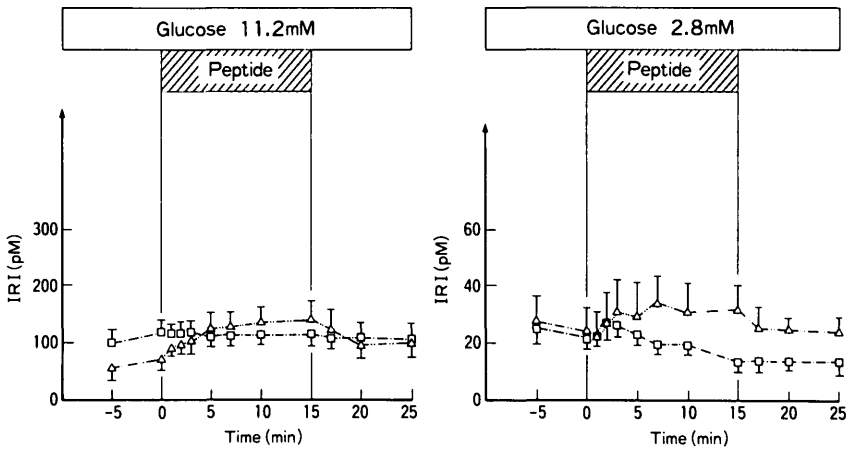

FIG. 4. Effect of 25 nM glucagonlike peptide I (GLP-I)-(1–37) (□) or 25 nM GLP-II-(1–34) (△) on immunoreactive insulin (IRI) secretion from perfused rat pancreas in 11.2 or 2.8 mM glucose. *Peptide* indicates GLP-I-(1–37) or GLP-II-(1–34). Values are means ± SE; *n* = 6.

by 0.25 or 2.5 nM of GLP-I-(7–36)-amide without any change in insulin release in the low glucose concentration may exclude the involvement of endogenous insulin (Table 1).

Somatostatin has been reported to be a mediator of the suppression of glucagon during hyperglycemia (22); in a recent study, GLP-I-(7–36)-amide significantly increased somatostatin secretion from the porcine pancreas (23). Although we did not analyze the change of somatostatin release in this study of the rat pancreas perfusion, we could not exclude that the inhibitory effect of GLP-I-(7–36)-amide on glucagon secretion was mediated by the stimulated somatostatin release.

GLP-I-(7–36)-amide and GLP-I-(7–37), which are truncated forms of full-sequence GLP-I-(1–37), have been reported in the lower intestine (9,10). Furthermore, plasma GLP-I-(7–36)-amide immunoreactivity to oral glucose in healthy volunteers has increased from 0.015 to 0.048 nM at 30 min after ingestion (9). The concentration of GLP-I-(7–36)-amide sufficient to stimulate insulin release in this study (0.025 nM) was comparable to that in peripheral circulation. Thus, GLP-I-(7–36)-amide may play an important role in the regulation of endogenous glucagon and insulin secretion in a reciprocal manner in the enteroinsular axis.

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