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Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets

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Summary. Glucagon-like peptide-1 and glucagon-like peptide-2 are encoded by the m-RNA of pancreatic preproglucagon. They show high conservation in different species and substantial sequence homology to glucagon. Because no definite biological activity of these peptides has been reported, we investigated the effect of synthetic C-terminally amidated glucagon-like peptide-1 [1–36] and synthetic human glucagon-like peptide-2 [1–34] with a free C-terminus on insulin release from isolated precultured rat pancreatic islets in the presence of glucose. Glucagon-like peptide-1 stimulates insulin release at 10 and 16.7 mmol/l glucose in a dose-dependent manner. Significant stimulation starts at 2.5 nmol/l in the presence of 10 mmol/l glucose and near maximal release is observed at 250 nmol/l, with approximately 100% increase over basal at both glucose concentrations. The peptide reaches 63% of the maximal stimulatory effect of glucagon. No stimulation occurs in the presence of 2.8 mmol/l glucose. Glucagon-like peptide-2 has no effect on insulin secretion at any glucose concentration tested. It is concluded that glucagon-like peptide-1, in contrast to glucagon-like peptide-2, exhibits a glucose-dependent insulinotropic action on isolated rat pancreatic islets similar to that of glucagon and gastric inhibitory polypeptide.

Key words: Glucagon-like peptide-1, glucagon-like peptide-2, insulin release, glucose dependency, isolated islets.

Pancreatic preproglucagon m-RNA, the precursor of pancreatic glucagon, has been cloned and sequenced from different species using recombinant DNA-techniques [1–5]. Two other peptides have been found arranged in tandem on the same m-RNA in close proximity C-terminal to the glucagon precursor: glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) comprising 36 and 34 amino acid residues, respectively. Both peptides show a different degree of sequence homology to pancreatic glucagon. The GLP-1 sequence is identical in the human, bovine and hamster precursor, whereas minor differences exist between the corresponding GLP-2 sequences. This substantial conservation in evolution and the close sequence homology to the glucagon molecule (GLP-1: 48% identical residues; GLP-2: 38%) indicates that GLP-1 and GLP-2 may play an important role of their own as hormones or local modulators. Recently they have been detected by specific radioimmunoassays in glucagonomas and in rat pancreatic islets [6], although they have not to date been isolated at the peptide level. Very little is known about a specific biological activity of these peptides. Hoosein and Gurd [7] could demonstrate a potent stimulation of rat hypothalamic and pituitary adenylate cyclase, whereas binding of glucagon to rat brain and liver membranes was not inhibited. GLP-1 may exert a weak stimulatory effect on exocrine pancreatic secretion in the rat [8].

Suggesting a role in carbohydrate metabolism, we investigated the effect of synthetic GLP-1 and GLP-2 on insulin release from isolated precultured rat pancreatic islets in the presence of glucose.

Material and methods

Pancreatic islets from fed male Wistar rats weighing 200–250 g were isolated by collagenase digestion of the pancreas [9]. The islets were kept in tissue culture medium 199 [10] for 24 h. This procedure in-

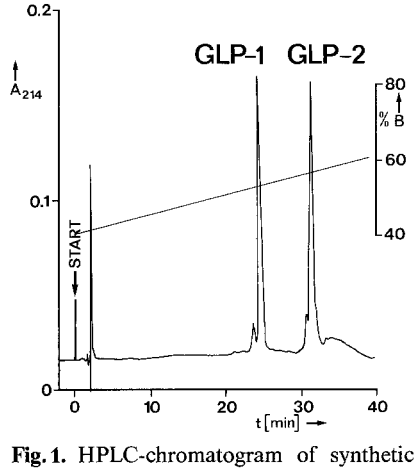

Fig. 1. HPLC-chromatogram of synthetic glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) (10 µg each, injected together) using the TFA/acetonitrile gradient system. Temperature: 40 °C; detection: 214 nm, sensitivity: 0.2 AU; flow 1.5 ml/min

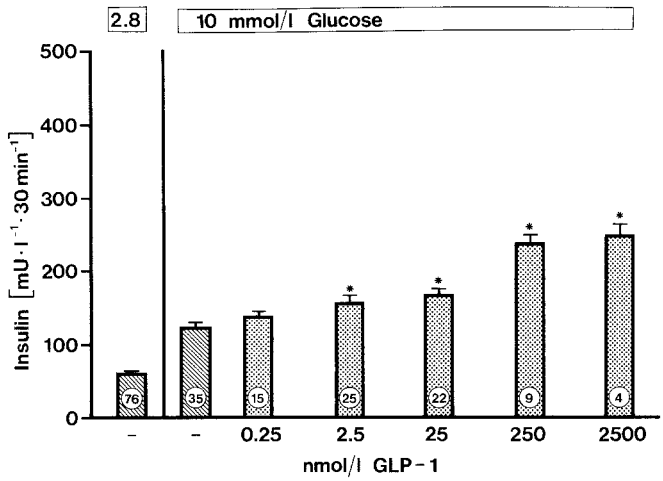

Fig. 2. Effect of glucagon-like peptide-1 on insulin release from isolated precultured rat pancreatic islets in the presence of 10 mmol/l glucose (right panel). For comparison basal release at 2.8 mmol/l glucose (left panel) is included; the number of experiments in each group is given in circle. ✻ = $p < 0.05$

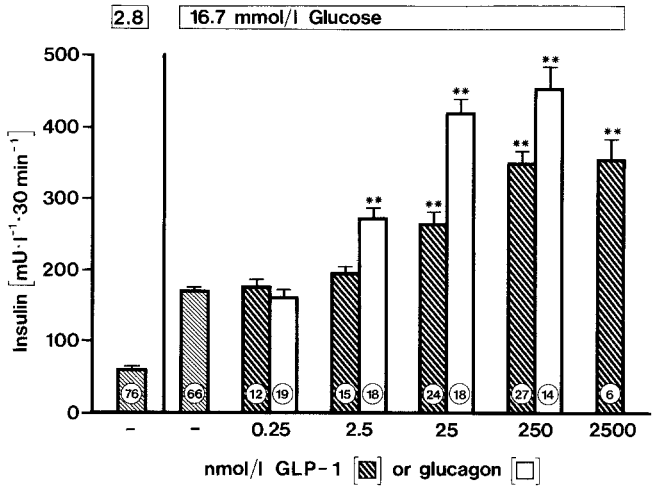

Fig. 3. Effect of glucagon-like peptide-1 (hatched bars) and glucagon (open bars) on insulin release from isolated precultured rat pancreatic islets in the presence of 16.7 mmol/l glucose (right panel). For comparison basal release at 2.8 mmol/l glucose (left panel) is included; the number of experiments in each group is given in circle; ✻✻ = $p < 0.01$

creases markedly the hormone sensitivity of isolated islets [11]. Static incubations of 10 islets per vial were performed in 2 ml oxigenized Krebs-Ringer Hepes buffer pH 7.4 containing 0.2% (w/v) bovine serum albumin and 1% (w/v) Hepes for 30 min in the presence of glucose at 2.8, 10 or 16.7 mmol/l. Synthetic GLP-1 (1-36, C-term.:-Arg-NH2) and GLP-2 (1-34, C-term.:-Arg-COOH, human sequence) (Peninsula Lab., Belmont, Calif., USA), were purified by high-performance liquid chromatography (HPLC) (Waters, Milford) on a reversed phase C-18 wide-pore column (Vydac RP 218) using a trifluoroacetic acid (TFA) / acetonitrile gradient system (buffer A: 0.1% TFA; buffer B: 0.1% TFA/29.9% water/70% acetonitrile). Islets were incubated with HPLC-pure peptides at concentrations of 0.25, 2.5, 25, 250 and 2500 nmol/l, respectively. Insulin release was measured by radioimmunoassay [12]. Results are expressed in $mU \cdot l^{-1} \cdot 30\ min^{-1}$ as mean ± SEM and were analyzed by the two-tailed Student's t-test for unpaired data adapted for multiple comparisons according to Holm [13]; $p < 0.05$ was considered to be significant.

Results

The HPLC-chromatogram of GLP-1 and GLP-2 (10 µg each, injected together) is shown in Figure 1. To avoid side-effects of impurities, only the middle portions of the symmetrical HPLC-peaks were used in biological experiments. The purity of the HPLC-rechromatographed peptides was estimated better than 99.5%. Figure 2 shows the effect of GLP-1 on insulin release from isolated precultured rat pancreatic islets in the presence of 10 mmol/l glucose and Figure 3 at 16.7 mmol/l glucose during a 30-min static incubation. Without peptide the insulin release raised by 100% in the presence of 10 mmol/l glucose compared to 2.8 mmol/l; another 50% increase was observed at 16.7 mmol/l glucose versus 10 mmol/l (Figs. 2 and 3). GLP-1 stimulated the release of insulin dose-dependently at 10 and 16.7 mmol/l glucose. No effect was observed at 2.8 mmol/l glucose (results not shown):

At 10 mmol/l glucose (Fig. 2), the stimulation reached statistical significance at 2.5 nmol/l GLP-1 (28% increase), whereas at 0.25 nmol/l there was only a tendency towards stimulation (12% increase). Near maximal release occurred at 250 nmol/l with 106% increase over basal.

In the presence of 16.7 mmol/l glucose (Fig. 3), the stimulation of insulin release by GLP-1 was similar with regard to percentage increase, ranging from 14% at 2.5 nmol/l to 103% at 2500 nmol/l. Maximal release was 38% higher than the corresponding value at 10 mmol/l glucose.

At 25 nmol/l, the incremental insulin release caused by GLP-1 reached 38% of the glucagon effect in the presence of 16.7 mmol/l glucose. Half-maximal stimulation was observed for GLP-1 at 25 nmol/l, for glucagon at 8-10 nmol/l. Maximal stimulation occurred with both peptides at 250 nmol/l. GLP-1 reached 63% of the effect produced by glucagon.

In contrast, GLP-2 did not influence insulin secretion at the same concentrations in the presence of 10 and 16.7 mmol/l glucose, as summarized in Table 1, nor at 2.8 mmol/l glucose (results not shown). At 10 mmol/l glucose, there was a slight tendency towards inhibition of insulin release (15% reduction) which did not reach statistical significance nor did it show a dose dependency.

Discussion

Only few data [7, 8] have been reported on biological effects of GLP-1 and GLP-2 which were characterized structurally as nucleotide sequences. The physiological significance of these findings remains unclear.

This study clearly shows that GLP-1 (1-36, C-terminally amidated), with 14 out of 29 homologous residues being the closer glucagon-related peptide (Fig. 4), stimulates insulin release from isolated precultured rat pancreatic islets in a dose- and glucose-dependent man-

Table 1. Effect of glucagon-like peptide-2 (GLP-2) on insulin release in isolated precultured rat pancreatic islets in the presence of 10 mmol/l and 16.7 mmol/l glucose

	Glucagon-like peptide-2 (GLP-2) added to the incubated islets (nmol/l)					
	–	0.25	2.5	25	250	2500
Insulin release ($mU \cdot l^{-1} \cdot 30\,min^{-1}$) at						
10 mmol/l glucose	122±7 ($n=35$)	n.d.	104±10 ($n=9$)	105± 9 ($n=10$)	116±15 ($n=7$)	n.d.
16.7 mmol/l glucose	172±6 ($n=66$)	n.d.	164±14 ($n=9$)	167±15 ($n=11$)	158±11 ($n=18$)	160±20 ($n=20$)

Values are expressed as mean±SEM, the differences between the groups are not statistically significant. n.d. = not determined; n = number of experiments

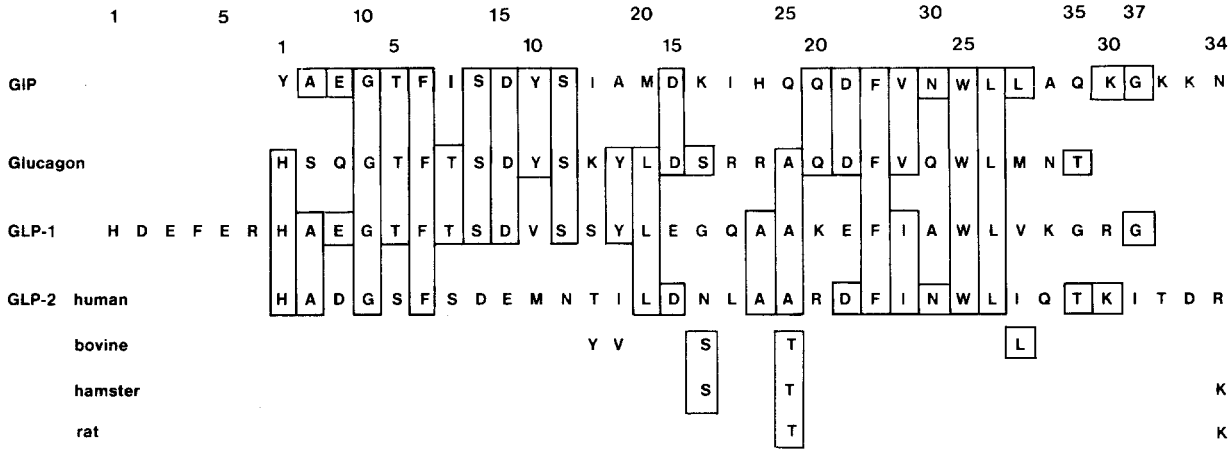

Fig. 4. Amino acid sequences of human GIP, residue 1-34 [17], glucagon, glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) [2-5]. The GLP-1 sequence is identical in the human, bovine, hamster and rat glucagon m-RNA precursor or gene, respectively; for GLP-2 the species-specific amino acid substitutions are marked

ner. A significant stimulation was observed at 2.5 nmol/l in the presence of 10 mmol/l glucose.

When compared with glucagon under identical incubation conditions, GLP-1 reaches approximately 40 to 60% of the increment in insulin release over a broad range of concentrations, but only in the presence of high glucose levels. Thus GLP-1 resembles the biological action of gastric inhibitory polypeptide (GIP), also named glucose-dependent insulinotropic polypeptide, which as the best-established "incretin" candidate stimulates insulin release in a strictly glucose-dependent manner [14-16]. Compared with GIP in a similar experimental setting [11], GLP-1 reaches approximately 40% of the maximal insulin response caused by GIP. Already at a concentration of 0.75-1.0 nmol/l, GIP shows a significant stimulation of insulin release, whereas the effect of GLP-1 starts at 2.5 nmol/l. The overall potency of GLP-1 in stimulating insulin release in this experimental system can be estimated as 30% compared to GIP.

GLP-1 resembles very closely the glucagon and GIP sequence in the N-terminal half of the molecule (Fig. 4) - 10 out of 15 N-terminal residues are identical in glucagon, 8 out of 11 in GIP, if position 7 of GLP-1 is aligned to position 1 of glucagon and GIP, respectively. This fact most probably explains the glucagon- and GIP-like action of the peptide on pancreatic islets. However, with regard to the N-terminal extension of GLP-1 beyond the glucagon-like sequence it appears unlikely that the action of GLP-1 is mediated through glucagon receptors [18]; in fact, it has been shown that GLP-1 does not bind to glucagon receptors in the liver [7], so one can speculate that GLP-1 may act on its own or on the GIP-receptor on the pancreatic B-cell which has been recently identified [19].

On the other hand it is yet not excluded that the N-terminally extended peptide of GLP-1 can be cleaved off by a trypsin-like enzyme. Due to its substantially closer sequence homology to glucagon and GIP, it is tempting to predict an even stronger glucagon- and/or GIP-like biological activity of the resulting GLP-1 [7-36] compared with the intact peptide - a hypothesis to be tested in further experiments.

In contrast to our findings, Ghiglione et al. [20] failed to demonstrate a glucagon-like activity of GLP-1 in vivo in rabbits on blood glucose or plasma insulin levels, even at pharmacological doses. Two reasons could explain these negative results: First, due to the lack of interaction with the glucagon receptor in the liver [7] no effect on hepatic gluconeogenesis can be ex-

pected. Secondly, the authors only investigated the effect of GLP-1 in fasting rabbits, i.e. in the absence of hyperglycemia, and their failure to stimulate insulin release at low glucose levels is in full agreement with our findings. It is also well established that other insulinotropic peptides like GIP do not stimulate insulin release at basal glucose concentrations [14, 16].

Whether the fact that the authors used the C-terminally non-amidated form of GLP-1 in their experiments could contribute to the lack of biological activity remains doubtful, since at present no regulatory peptide has been found to possess - Arg-NH_2 at the C-terminus. Moreover, for the biological activity within the glucagon family, the N-terminal amino acid residues seem to be essential [18].

Human GLP-2, which possesses 11 out of the 29 amino acid residues of glucagon at homologous positions (Fig. 4), did not augment the secretion of insulin at any of the investigated glucose concentrations. This could be due to the considerably lower degree of sequence homology to glucagon, especially with regard to the N-terminal part of the molecule (residues 4 to 13) which is highly conserved within the glucagon family of peptides. In addition, our experiments were carried out with synthetic human GLP-2, which differs from the corresponding recently cloned rat sequence in two residues [5]. A specific biological activity of this peptide has still to be identified.

A recent study of the in vivo posttranslational processing of the glucagon precursor in rat pancreatic islets [21] leads to the suggestion that a tryptic-like cleavage does not occur between the GLP-1 and GLP-2 sequences. Instead it was shown that both sequences are secreted mainly as one "major proglucagon fragment" with very little, if any, GLP-1 and GLP-2 as single entities. These observations raise the question whether this type of posttranslational processing is specific for the pancreatic islet or uniform in other glucagon-containing tissues like the gut. If differential processing occurs within the gut, GLP-1 (1-36) could well qualify as a member of the "incretins" which augment insulin release after an oral glucose load [22]. However, any definite physiological role of these glucagon-like peptides remains to be elucidated.

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