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How glucagon-like is glucagon-like peptide-1?

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Summary. Although glucagon-like peptide-1 has the appearance of a glucagon-homologue that may be co-secreted with glucagon, synthetic glucagon-like peptide-1-(1–37) does not significantly affect plasma glucose and insulin concentrations when administered at high doses (100 and 400 μg) to cortisone-pretreated rabbits. This synthetic preparation thus lacks the primary metabolic effect of glucagon at the doses tested. An intra- or extra-pancreatic role of glucagon-like peptide-1 has yet to be discovered.

Key words: Glucagon-like peptide-1, glucose, insulin, glucagon, bioassay.

The nucleotide sequences of cDNA derived from hamster [1] and ox [2] glucagon mRNA and the human pre-proglucagon gene [3] show that pre-proglucagon contains two further glucagon-like peptides (GLP-1 and -2) C-terminal to the glucagon sequence. The amino-acid sequence of GLP-1 is completely conserved between the three mammalian species studied and shows a high degree of homology with similar deduced amino-acid sequences in angler-fish pre-proglucagons [4]. It is defined by delimiting pairs of basic amino-acid residues, that form potential cleavage points. GLP-1 thus has the appearance of a highly conserved biologically active peptide that may be co-secreted with glucagon, and it is possible that it has a modulating role in carbohydrate metabolism. In the first instance we have tested synthetic GLP-1 for its effect on plasma glucose and insulin concentrations in rabbits.

Material and methods

Synthetic GLP-1-(1–37) was synthesized by a solid phase method [5] (Bachem, Torrance, CA, USA). It was tested for its effect on plasma glucose and insulin by a modification of the twin cross-over bioassay for glucagon [6], using crystalline glucagon (Novo Industri, Copenhagen, Denmark) as a standard. Twelve rabbits were injected (25 mg subcutaneously) on day 0 with cortisone acetate injection (British Pharmacopoeia; Cortistab, The Boots Company, Nottingham, UK). The assay was performed on days 2 and 3, the rabbits being deprived of food for 18 h before each part of the assay, but with free access to water. The peptides were dissolved immediately before each experiment in 1.6% (vol/vol) aqueous glycerine containing 0.2% (wt/vol) phenol, adjusted to pH 3 with HCl.

The rabbits were randomized into four groups of three each, and they received a subcutaneous injection of 1 ml of diluent, as a control, followed after 60 min by either GLP-1 (400 μg or 100 μg) or glucagon (24 μg or 6 μg) in the same volume. Blood samples (0.3 ml) were taken from a marginal ear vein at times −40, 0, 20 and 60 min in relation to both the diluent and peptide injections. The next day, the experiment was repeated with a twin cross-over, so that rabbits that had received the higher dose of glucagon now received the lower dose of GLP-1. The blood samples were collected into fluoride-oxalate centrifuge tubes, containing dried aprotinin (200 Kallikrein Inhibitor Units; Trasylol, Bayer, Wuppertal, FRG), and centrifuged immediately at 1600 *g* for 10 min. The plasma was frozen on solid CO_2 and stored at −20 °C. Plasma glucose concentrations were determined with a glucose analyzer (No. 2, Beckman Instruments, Fullerton, California, USA), and immunoreactive insulin was determined by radioimmunoassay [7]. Results were analysed by Student's paired t-test on log transformed data.

Results

Plasma glucose and immunoreactive insulin concentrations obtained at 20 min after the injection of diluent controls, GLP-1 at doses of 400 μg and 100 μg and glucagon at doses of 24 μg and 6 μg are shown in Figure 1. Glucagon produced the expected significant increases in plasma glucose and insulin concentrations, whereas GLP-1 had no effect on plasma glucose at either dose. This was also the case at 60 min after injection. The slight increases in the mean plasma insulin concentration seen 20 min after GLP-1 injections persisted at 60 min with the 400 μg dose, but not with the 100 μg dose, and were not statistically significant on a paired basis.

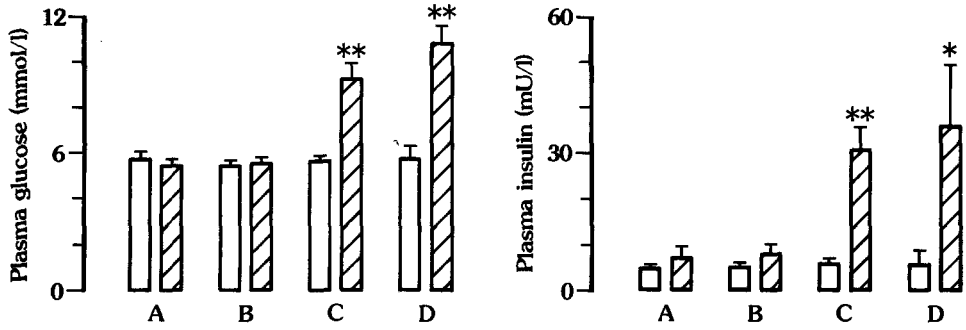

Fig. 1. Plasma glucose and insulin concentrations in rabbits 20 min after injection of ▨ ($n = 6$) (A) GLP-1 100 μg, (B) GLP-1 400 μg, (C) glucagon 6 μg, (D) glucagon 24 μg, compared with their respective control values 20 min after diluent injections □. Bars represent mean and SEM; ** $p < 0.001$, * $p < 0.05$

Table 1. Amino-acid sequences of GLP-1 and pancreatic glucagon [1]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
GLP-1	His	-Asp	-Glu	-Phe	-Glu	-Arg	-His	-Ala	-Glu	-Gly	-Thr	-Phe	-Thr	-Ser	-Asp	-Val	-Ser	-Ser	-Tyr-
Glucagon							His	-Ser	-Gln	-Gly	-Thr	-Phe	-Thr	-Ser	-Asp	-Tyr	-Ser	-Lys	-Tyr-
							1	2	3	4	5	6	7	8	9	10	11	12	13
	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	
GLP-1	-Leu	-Glu	-Gly	-Gln	-Ala	-Ala	-Lys	-Glu	-Phe	-Ile	-Ala	-Trp	-Leu	-Val	-Lys	-Gly	-Arg	-Gly	
Glucagon	-Leu	-Asp	-Ser	-Arg	-Arg	-Ala	-Gln	-Asp	-Phe	-Val	-Gln	-Trp	-Leu	-Met	-Asn	-Thr			
	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29			

Discussion

Despite the sequence homology between GLP-1 and glucagon (14 out of 29 comparable residues), GLP-1 does not affect plasma glucose concentrations, even at the high doses used in this study. It must therefore possess considerably less than 1% of the hyperglycaemic activity of glucagon, 2 μg doses of which produce detectable responses in the assay used [6]. The full amino-acid sequences of GLP-1 and glucagon are shown in Table 1. The similarity between glucagon and GLP-1 is greatest in the first nine residues of glucagon, where there are seven amino-acid identities; but GLP-1 has a further six amino-acid residues extending its N-terminal region beyond that of glucagon. As N-terminally extended forms of glucagon do not interact with hepatic glucagon receptors [8], the N-terminal extension of GLP-1 is only one of several structural features that may prevent binding to glucagon receptors. However, this study shows that GLP-1 does not produce a hyperglycaemic effect by an alternative mechanism. There remains the possibility that GLP-1 has a weak effect on insulin release, requiring higher doses or a larger number of animals than were available in this study for a significant release to be demonstrated. While this points to a possible role for GLP-1 within the islet, GLP-1 receptors have yet to be identified in any tissue, and any definite intra- or extra-pancreatic role remains to be discovered.

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