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Glucagon-like peptide 1: a potent glycogenic hormone

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Abstract

GLP-1(7–36)amide is an insulinotropic peptide derived from the intestinal post-translational proglucagon process, the release of which is increased mainly after a carbohydrate meal; also, its anti-diabetogenic effect in normal and diabetic states has been reported. In this study, GLP-1(7–36)amide stimulates the formation of glycogen from glucose in isolated rat hepatocytes, such a glycogenic effect being achieved with physiological concentrations of the peptide. The GLP-1(7–36)amide-induced glycogenesis is abolished by glucagon, and it is accompanied by stimulation of the glycogen synthase *a* activity and by a decrease in the basal and glucagon-stimulated cyclic AMP content. These findings could explain, at least in part, the GLP-1(7–36)amide insulin-independent plasma glucose lowering effect.

Key words: GLP-1; Hepatic glycogen; Glycogen-synthase; cAMP

1. Introduction

Glucagon-like peptide 1 (GLP-1) is an intestinal post-translational proglucagon product found in four variants: proglucagon-78–108, or GLP-1(7–37), proglucagon-72–108, or GLP-1(1–37), and their respective amidated forms. The short forms, major products of the intestinal proglucagon molecule released during glucose absorption, are glucose-dependent insulinotropic (see [1] for review). The reported lack of effect of GLP-1(1–37) and GLP-1(7–37) on cyclic AMP formation, and the absence of glucagon-like effects (glycogenolysis and gluconeogenesis) on rat hepatic glucose metabolism [2–4], supported the general believe that, except in the fish [5], GLP-1 was without effect on the carbohydrate liver metabolism (see [6] for review). Yet, the recent reported anti-diabetogenic effect of GLP-1(7–36)amide and GLP-1(7–37) [7,8] prompted us to explore the liver as a possible site for extrapancreatic effects of these peptides on glucose disposal. We have studied the effect of GLP-1s on glycogen synthesis in isolated rat hepatocytes.

2. Materials and methods

2.1. Animals

Fed Wistar rats, weighing 150–200 g, and kept on a standard pellet diet (UAR, Panlab, Spain) with tap water ad libitum.

2.2. Chemicals

Synthetic GLP-1 (1–36)amide (lot no. 018862), GLP-1(7–36)amide (lot no. 010448), GLP-1(1–37) (lot no. 031790) and GLP-1(7–37) (lot no. 019860), were obtained from Peninsula Lab. Inc. (Belmont, CA). Crystalline pork glucagon (lot no. 2587 N6–46) was a gift from Eli Lilly Co. (Indianapolis, IN). Rat insulin (lot no. 220891) was from Novo BioLabs (Bagsvaerd, Denmark). BSA (Fraction V) was from Sigma Chemical Co. (St. Louis, MO), and Collagenase A from *Clostridium histolyticum* was from Boehringer-Mannheim GmbH (Mannheim, Germany). Protein assay kits (Bio-Rad protein assay) were from Bio-Rad Laboratories (Munich, Germany); Rianen cAMP [^{125}I]RIA kits were from DuPont Co. (Brussels, Belgium). D-[U-^{14}C]glucose and D-[5-^{3}H]glucose were from Amersham (Little Chalfont, UK), and Ultima Gold scintillation liquid was from Packard (Groningen, The Netherlands).

2.3. Glycogen synthesis studies

We have examined the incorporation of D-[U-^{14}C]glucose into cellular glycogen accordingly to the procedure described by Fleig et al. [9], in isolated hepatocytes prepared as in Hue et al. [10]. Cells were first resuspended in Krebs-Ringer bicarbonate buffer (118 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, and 25 mM $HNaCO_3$), pH 7.4 (KRB), containing 10 mM D-glucose, and then preincubated for 30 min at 37°C; after, 1×10^6 hepatocytes were incubated for 30 min in 300 μl KRB, containing 0.05% BSA (bovine serum albumin), with 0.75 μCi [U-^{14}C]glucose at 20 mM D-glucose (or otherwise stated), in the absence or presence of insulin, GLP-1s and/or glucagon.

2.4. Glycogen-synthase a and -phosphorylase a activities

Isolated overnight fasted rat hepatocytes (4×10^6), previously preincubated for 15 min at 37°C in KRB containing 5.6 mM D-glucose, were incubated for 10 min in 100 μl KRB, containing 0.05% BSA and 16.7 mM glucose, in the absence or presence of 10^{-9} M GLP-1(7–36)amide, 10^{-9} M insulin and/or 10^{-9} M glucagon; the cells were immediately homogenized and frozen until the enzymes activities were assayed as in Hue et al. [10] except that the final glycogen extraction was as in Fleig et al. [9].

2.5. Cyclic AMP

We have examined the cyclic AMP content in rat hepatocytes after 3 min incubation at 37° C, in the presence of 10^{-8} M amidated GLP-1s or insulin, alone or in combination with 10^{-9} M glucagon, while in the absence or presence of 1 mM IBMX. For that, after a preincubation period as in for glycogen synthesis, cells (3×10^5) were incubated in 200 μl KRB buffer, pH 7.4, containing 10 mM D-glucose and 0.05% BSA, for 3 min at 37°C, in the absence or presence of IBMX (1.0 mM) and the given peptides. The incubation was stopped by the addition of 800 μl cold 81.25% ethanol; after mixing, each sample was centrifuged, and the corresponding supernatant was evaporated and reconstituted for cyclic AMP radioimmunoassay.

2.6. Statistical study

Results are expressed as mean ± S.E.M. Statistical significance of the increments was assessed by the Student's *t*-test. Analysis of variance was also performed when appropriate.

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3. Results

3.1. Glycogen synthesis

As shown in Fig. 1, the incorporation of glucose into hepatocyte glycogen, after 30 min incubation, was significantly increased by GLP-1(7–36)amide ($P < 0.001$, $F = 17.5$, df = 50, as determined by analysis of variance) and by GLP-1(1–36)amide ($P < 0.001$, $F = 19.9$, df = 51), as well as by insulin ($P < 0.001$, $F = 19.5$, df = 91). The maximal increment in glycogen formation by GLP-1(7–36)amide or GLP-1(1–36)amide was achieved at 10^{-10} M, whereas by insulin it was at 10^{-8} M. The lower concentration at which a significant effect was observed was 10^{-12} M for GLP-1(7–36)amide ($P < 0.01$, $R = 2$, by the Newman-Keuls test) and for GLP-1(1–36)amide ($P < 0.01$, $R = 2$) while, for insulin, it was 10^{-10} M ($P < 0.01$, $R = 4$); the ED_{50} was close to 10^{-12} M for both amidated GLP-1 forms, and to 10^{-10} M for insulin. The values reached with 10^{-12} M to 10^{-9} M GLP-1(7–36)amide were significantly higher than those with insulin at the same respective doses (10^{-12} M and 10^{-11} M, $P < 0.001$, df = 16; 10^{-10} M and 10^{-9} M, $P < 0.05$, df = 18 and 27, by the Student's t-test); essentially, the same was observed with GLP-1(1–36)amide (10^{-12} M and 10^{-11} M, $P < 0.001$, df = 19 and 17; 10^{-10} M, $P < 0.01$, df = 22; 10^{-9} M, $P > 0.05$, df = 29).

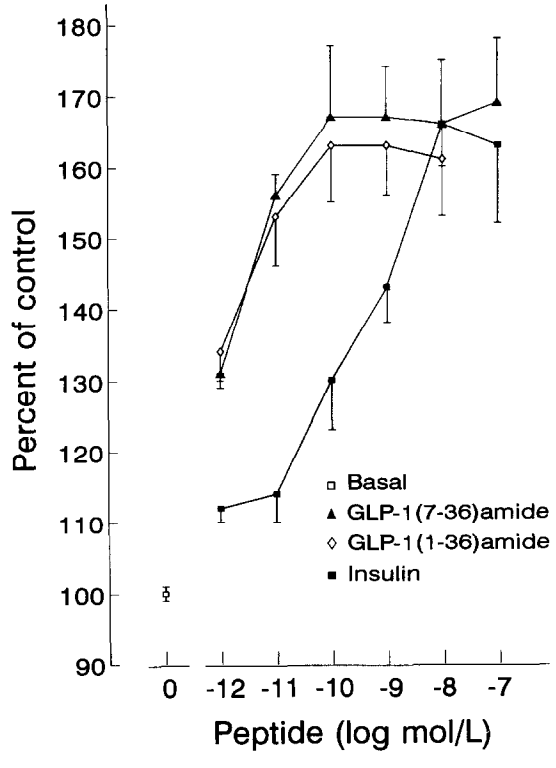

Fig. 1. Incorporation of D-[U-^{14}C]glucose into rat hepatocyte glycogen. Effect of rat insulin, GLP-1(7–36)amide and GLP-1(1–36)amide. Mean values (± S.E.M.) correspond to that of individual observations from 2–6 separate hepatocyte preparations, each set up in, at least, duplicate. Values are expressed as the percentage of the mean value obtained in the absence of added peptides within the same experiment (6.4 ± 0.3 nmol/mg in 30 min, $n = 18$).

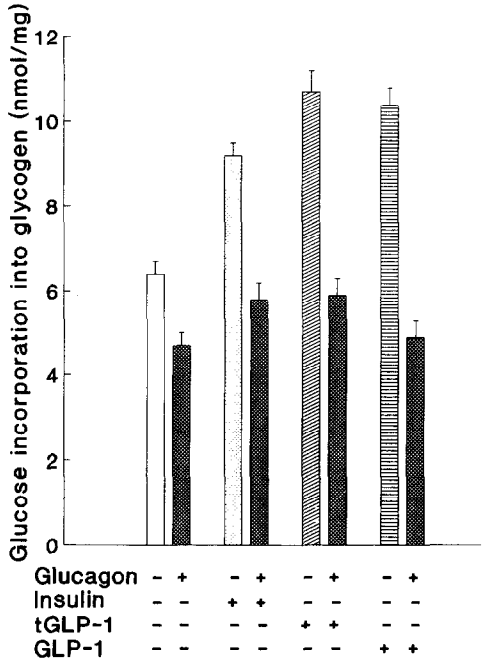

Fig. 2. Effect of 10^{-9} M glucagon alone and in combination with 10^{-9} M other peptides on glycogen synthesis in rat hepatocytes. Mean values (± S.E.M.) correspond to that of individual observations from 2–6 separate hepatocyte preparations, each set up in, at least, triplicate. Values are expressed as nmol of glucose incorporated into glycogen, per mg cell protein, in 30 min.

As shown in Fig. 2, glucagon at 10^{-9} M inhibited the basal glycogen formation ($P < 0.01$, df = 6, by the Student's t-test) and abolished that induced by 10^{-9} M insulin ($P < 0.001$, df = 24), GLP-1(7–36)amide ($p < 0.001$ d.f = 17) or GLP-1(1–36)amide ($P < 0.001$, df = 15).

A significant glycogenic effect ($P < 0.05$ or more) of both amidated GLP-1 forms (10^{-9} M), as well as that of insulin, was also observed when glucose in the medium was lowered to 5 and 10 mM glucose (Fig. 3).

When glycogen synthesis was stimulated by combined submaximal doses (10^{-11} M) of insulin (130 ± 11% of basal, $n = 16$) and either GLP-1(7–36)amide (156 ± 13%, $n = 9$) or GLP-1(1–36)amide (156 ± 34%, $n = 9$), the effect was additive (190 ± 18%, $n = 17$ and 185 ± 32%, $n = 18$, respectively), whereas maximal effective doses (10^{-8} M), tested in combination, did not elicit any further increment.

The non-amidated forms of GLP-1 were tested at 10^{-8} M, and they also showed to elicit a significant increment in glycogen synthesis, being 125 ± 9% of basal ($n = 27$, $P < 0.01$) and 134 ± 7% ($n = 25$, $P < 0.001$), for GLP-1(1–37) and GLP-1(7–37), respectively, both values lower than that achieved by 10^{-8} M insulin (160 ± 12%, $n = 28$) tested in parallel in seven individual experiments.

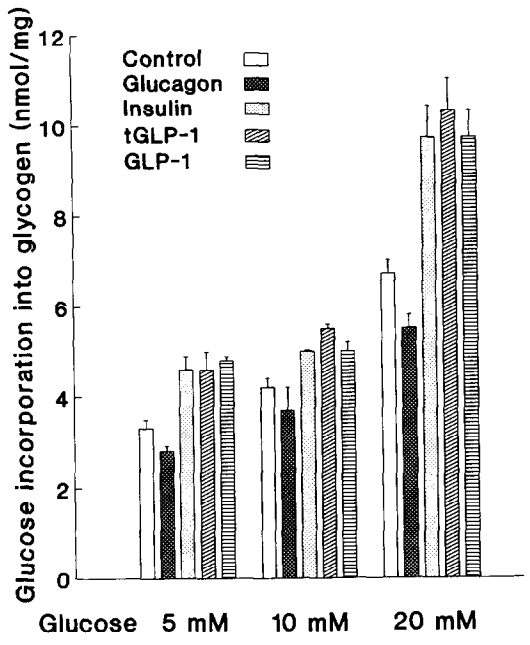

Fig. 3 Effect of glucose on the incorporation of D-[U-^{14}C]glucose into rat hepatocyte glycogen. Peptides were all added at 10^{-9} M. Mean values (± S.E.M.) correspond to one experiment set up in triplicate. Values are expressed as nmol of glucose incorporated into glycogen, per mg cell protein, in 30 min.

3.2. Glycogen-synthase a and -phosphorylase a activity

In 10 min incubation, GLP-1(7–36)amide, at 10^{-9} M, significantly increased ($P < 0.001$, $n = 10$ individual experiments, each set up in triplicate) the synthase *a* activity to 154 ± 9% of the value obtained in the absence of peptide within the same experiment (0.148 ± 0.017 units/g of protein, $n = 10$), and this response was higher ($P < 0.05$) than that of 10^{-9} M insulin (124 ± 7%, $n = 5$ experiments run in parallel); 10^{-9} M glucagon alone inhibited the basal synthase *a* activity to 77 ± 7%, $n = 6$, $P < 0.05$, and when in combination with 10^{-9} M GLP-1(7–36)amide or insulin, the respective values obtained were not different from the basal (90 ± 8% of basal and 89 ± 8%, $n = 6$ both groups). No significant changes of the basal value (25 ± 3, $n = 10$, unit/g of protein) were detected in the phosphorylase *a* activity except with 10^{-9} M glucagon (118 ± 4%, $n = 10$, $P < 0.01$; the values obtained when glucagon was combined with 10^{-9} M GLP-1(7–36)amide or insulin, were not different from that of the basal (106 ± 3% of basal and 102 ± 8, $n = 6$ both groups).

3.3. Cyclic AMP

It was observed (Table 1) that, in the absence of IBMX, the basal and glucagon-stimulated hepatocytes cyclic AMP content were significantly reduced by the presence of GLP-1(7–36)amide or GLP-1(1–37)amide, as it occurred with insulin, while that was not observed when IBMX was present. The non-amidated peptides, tested in the absence of IBMX, also significantly reduced the glucagon-stimulated cyclic AMP content.

4. Discussion

GLP-1(7–36)amide and GLP-1(1–37)amide showed to have in isolated rat hepatocytes a potent glycogenic action which, at least in the case of GLP-1(7–36)amide, was accompanied by an increase in glycogen synthase *a* activity, being both effects abolished by glucagon; the non-amidated forms also had, to some extent, a glycogenic effect. Furthermore, these peptides reduced, in the absence of IBMX, the glucagon-stimulated hepatocyte cyclic AMP content; in addition, the amidated forms also reduced the basal content.

These results indicate a direct glycogenic effect on rat hepatocytes of the four variants of GLP-1; among them, at least GLP-1(7–36)amide, which is known to be released after a carbohydrate meal, exerted this effect at

Table 1
Effect of GLP-1 peptides upon cyclic AMP production in isolated fed rat hepatocytes

Peptides 10^{-8} M	Glucagon 10^{-9} M	Cyclic AMP production, percentage of control					
		No IBMX	P vs. a	P vs. b	IBMX	P vs. a	P vs. b
–	–	100.0 ± 6.3 (10)[a]			100.0 ± 12.0 (8)[a]		
–	+	170.3 ± 6.7 (10)[b]	<0.001		580.3 ± 67.1 (8)[b]	<0.001	
Insulin	–	71.8 ± 4.6 (6)	<0.05		88.1 ± 7.4 (8)	>0.1	
Insulin	+	119.0 ± 12.4 (6)	<0.1	<0.01	568.4 ± 38.0 (8)	<0.001	>0.1
GLP-1(7–36) amide	–	74.0 ± 5.7 (7)	<0.05		92.0 ± 6.4 (7)	>0.1	
GLP-1(7–36) amide	+	122.4 ± 11.0 (7)	<0.1	<0.01	487.0 ± 25.1 (8)	<0.001	>0.1
GLP-1(1–36) amide	–	67.8 ± 4.2 (6)	<0.01		95.0 ± 8.7 (8)	>0.1	
GLP-1(1–36) amide	+	129.4 ± 13.2 (5)	<0.1	<0.01	543.3 ± 40.7 (6)	<0.001	>0.1
GLP-1(7–37)	–	114.4 ± 10.0 (5)	>0.1				
GLP-1(7–37)	+	135.4 ± 13.9 (5)	<0.01	<0.05			
GLP-1(1–37)	–	99.4 ± 9.2 (5)	>0.1				
GLP-1(1–37)	+	134.6 ± 10.6 (5)	<0.05	<0.05			

The data (mean ± S.E.M., followed by the number of samples in parenthesis) are expressed as the percent of the respective mean control value in the absence (2.0 pmol/mg) or presence of IBMX (2.7 pmol/mg).

physiological concentrations [11–13]. The increase in glycogen synthesis was accompanied by a stimulation of the synthase *a* activity and by a decrease of the cyclic AMP content only in the absence of IBMX, which indicates that a stimulation of a cyclic AMP phosphodiesterase activity occurs; hence, the last steps in the mechanism of the glycogenic effect of GLP-1s could be the same as that of insulin. On the other hand, we have evidence for the presence of specific binding of the amidated peptides can be detected in hepatocytes plasma membranes, which does not show binding with insulin or glucagon [14]; thus, if the effect of GLP-1s in the liver occurs through specific receptors, those could be different to that described for GLP-1(7–36)amide in pancreatic B cells [15], in which the insulinotropic action of the peptide is associated with an activation of adenylate cyclase [16,17]. We believe that GLP-1(7–36)amide may play a physiological and physiopathological role (i.e. diabetes mellitus) at the level of the glucose storage in the liver, which could explain, at least in part, its insulin-independent plasma glucose lowering effect.

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