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Potent glycogenic effect of GLP-1(7–36)amide in rat skeletal muscle

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Summary GLP-1(7–36)amide is an intestinal post-translational proglucagon product released mainly after carbohydrate ingestion, the glucose dependent insulinotropic and antidiabetogenic actions of which have been documented. In this work, by exploring whether GLP-1(7–36)amide has any effect on the glucose metabolism of the muscle, we have observed that this peptide, at physiological concentrations, exerts in this tissue an increment of the D-[U-^{14}C]glucose incorporated into glycogen, which is accompanied by an increase in the glycogen synthase *a* activity; also, it stimulates both glucose oxidation and lactate formation. These data indicate that the skeletal muscle is one of the target tissues for GLP-1(7–36)amide, where its insulin-like effect explains, at least in part, its plasma glucose lowering action; thus, GLP-1(7–36)amide may well be implicated in the physiological control of glucose homeostasis after meals, not only by acting as an incretin, but also by directly promoting glucose disposal. [Diabetologia (1994) 37: 1163–1166]

Key words GLP-1(7–36)amide, glycogenesis, skeletal muscle.

The glucose-dependent insulinotropic proglucagon-derived peptide GLP-1(7–36)amide, which is released mainly after glucose [see Ref. 1, for review] has an antidiabetogenic action in normal and diabetic states [2]. In this work we have explored whether GLP-1(7–36)amide could have insulin-like effects on glucose metabolism in the muscle.

Materials and methods

Wistar rats, weighing 200–250 g and kept on a standard pellet diet (UAR, Panlab, Barcelona, Spain) with tap water ad libitum, were used. For glycogen synthesis studies, the rat was killed by a blow to the head, and the two intact soleus muscles were removed, then individually attached by the tendon to a steel clip [3]. After rinsing in saline solution, they were immediately preincubated at 37 °C in 1.5 ml Krebs Ringer buffer (KRB) containing 1 % bovine serum albumin (BSA) and 5 mmol/l D-glucose for 30 min, while undergoing continuous gentle shaking. Preincubation was followed by incubation for 60 min, for which the muscles were transferred to another vial containing the same medium except for the presence of 0.35 μCi D-[U-^{14}C]glucose and 0.3 μCi D-[5-^{3}H]glucose (both from Amersham, Little Chalfont, Bucks., UK), and either without (control) or with different concentrations of GLP-1(7–36)amide (Peninsula Lab. Inc., Belmont, Calif., USA) or rat insulin (Novo-Nordisk, Bagsvaerd, Denmark). During the preincubation and incubation, an atmosphere of O_2/CO_2 (95 : 5) was maintained in the vials sealed with a rubber stopper. Paired soleus muscles from one animal were used in each experiment, using one of the pair as control (absence of peptide or otherwise stated). At the end of the incubation period, each muscle was quickly washed in the same ice-cold media except for the absence of radioactivity and BSA. To further control the effect of GLP-1(7–36)amide on glycogen synthesis, an equimolar amount (10^{-10} mmol/l) of the peptide was pre-treated with an excess of a C-terminal glucagon-like peptide 1 (GLP-1) antiserum (no. 2135, a gift from Dr. J. J. Holst, Copenhagen, Denmark), for 4 days at 4 °C; the degree of GLP-1(7–36)amide bound (mean ± SEM; 100 ± 1, $n = 7$) was measured

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Abbreviations: KRB, Krebs-Ringer bicarbonate buffer, pH 7.4; EDTA, ethylenedinitrilo tetraacetic acid, disodium salt dihydrate; BSA, bovine serum albumin.

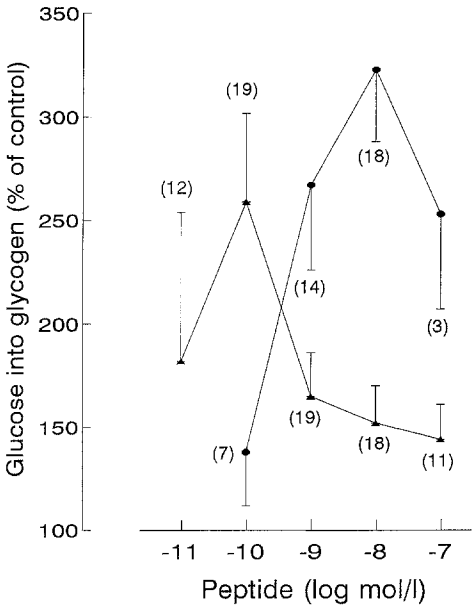

Fig. 1. Incorporation of D-[U-^{14}-C]glucose into cellular glycogen. Mean values (± SEM) correspond to the number of experiments, given in parentheses, for each peptide concentration. Values are expressed as percent of the control value, 2.3 ± 0.2 nmol glucosyl units/mg protein, $n = 121$, in 60 min. One glucosyl unit indicates one glucose moiety incorporated into glycogen

in parallel samples containing, in addition, tracer amounts of [^{125}I]-GLP-1(7–36)amide.

Radioactive glycogen content. To determine the D-[U-^{14}C]glucose incorporated into glycogen, the procedure described by Cuendet et al. [4] was basically followed; each muscle was extracted in 1 ml 1N NaOH for 10 min at 70 °C; after an aliquot sample (10 µl) had been taken for determination of total protein content by the Bradford method using BSA as standard (Bio-Rad protein assay, Bio-Rad, Munich, Germany), glycogen was precipitated with 85 % (v/v final) ethanol at 4 °C, in the presence of previously added carrier unlabelled glycogen (100 µg/ml, final). The precipitate was then sedimented by centrifugation, redissolved in 0.5 ml water, and the ^{14}C content measured in 5 ml scintillation liquid (Ultima Gold, Packard, Groningen, The Netherlands).

$^{14}CO_2$, ^3HOH and lactate production. $^{14}CO_2$, ^{3}HOH and lactate were measured simultaneously in most of the experimental samples used to study glycogen synthesis. The basic procedure described elsewhere [5] was followed; in brief, two cups with cellulose Whatman paper, one of which contained 0.5 ml 0.1 mol/l HCl for ^{3}HOH uptake, were hung from the rubber stopper during the muscle's 60-min incubation period. Then, 250 µl hyamine hydroxide (Hopkins & Williams, Chendwell Heath, UK) was injected into the second cup, for $^{14}CO_2$ uptake, and after 3 min at 4 °C, the muscle was removed and the cups subjected to a 30-min incubation at 37 °C followed by 60 min at 25 °C; after which the cups were separately placed in 5 ml of scintillation liquid for ^{3}H and ^{14}C content measurements. To control the ^{3}HOH recovery, a vial with 1.5 ml of the same medium containing 4 nCi ^{3}HOH (NEN Dupont Co., Brussels, Belgium), but containing no muscle, followed the whole procedure in each experimental set. Double-channel counting indicated the absence of cross-contamination. The medium was saved for lactate content determination, which was enzymatically assayed.

Glycogen synthase a and -phosphorylase a activities. Paired soleus muscles from one animal were used in each experiment, with one of the pair as control (absence of peptide). Muscles were preincubated for 30 min at 37 °C in 1.5 ml KRB containing 1 % BSA and 5 mmol/l D-glucose; preincubation was followed by 10 min incubation in the same medium except for the presence of 16.7 mmol/l D-glucose, and in the absence (control) and presence of rat insulin (10^{-8} mol/l) or GLP-1(7–36)amide (10^{-10} and 10^{-9} mol/l). At the end of the incubation period, each muscle was placed in liquid nitrogen and, when frozen, broken into particles by a steel hammer blow; immediately the tissue was resuspended in 500 µl ice-cold 50 mmol/l glycylglycine, pH 7.4, containing 0.5 % glycogen and 35 mmol/l EDTA, and then subjected to further homogenization by Polytron (Ystral 1500, Göttingen, Germany). After debris was removed by sedimentation at 200 *g* for 2 min, the supernatant was divided into two parts: one (30 µl) was used for cyclic AMP determination and, to the other (400 µl), 100 mmol/l final NaF was added for glycogen synthase *a* and -phosphorylase *a* activity determinations, which were assayed by the method of Hue et al. [6] except for the final glycogen extraction which was peformed as described by Fleig et al. [7].

Adenylate cyclase activity. The homogenized muscle aliquot samples taken during several of the experiments performed to study glycogen synthase *a* and glycogen phosphorylase *a*, were treated with 65 % (final) ethanol and, after centrifugation, the supernatants were evaporated and reconstituted for cyclic AMP assay (RIANEN cAMP [^{125}I]RIA Kits, Dupont Co., Brussels, Belgium).

Statistical analysis

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

Results

Figure 1 shows the dose-dependent effect of GLP-1(7–36)amide and of insulin on glycogen formation in rat soleus muscle. GLP-1(7–36)amide significantly increased the incorporation D-[^{14}C]glucose into glycogen ($p < 0.001$, $df = 157$, as determined by analysis of variance), as it did insulin ($p < 0.001$, $df = 83$). The maximal increment induced by GLP-1(7–36)amide was detected at 10^{-10} mmol/l, and although significant increments over basal ($p < 0.05$ or less) were still observed at 10^{-9}–10^{-7} mol/l, those were lower than that attained at 10^{-10} mol/l ($p < 0.05$ or less). As each value was obtained in individual experiments using paired muscle from the same rat as control without peptide, a further series of experiments was performed whereby one of the two muscles was incubated in the presence of GLP-1(7–36)amide at 10^{-10} mol/l, and the other at 10^{-9} mol/l. The $10^{-10}/10^{-9}$ ratio value was 1.67 ± 0.42 ($n = 7$), which is not different from that of the mean values of the two concentrations obtained in different experiments (1.57 ± 0.33, $n = 19$). When the equimolar amount of GLP-1

Table 1. Effect of GLP-1(7–36)amide and of insulin, on glucose oxidation ($^{14}CO_2$) and utilization (^{3}HOH formation) and lactate release, during 60 min incubation. Data are expressed as percent of the control paired value. Control values: $^{14}CO_2$, 153 ± 14, n = 106, pmol glucose/mg protein; ^{3}HOH, 43 ± 5, n = 106, nmol glucose/mg protein; lactate, 89 ± 4, n = 106, nmol/g protein

Peptide	Mol/l	n	$^{14}CO_2$ (% of control)	^{3}HOH (% of control)	Lactate (% of control)
GLP-1	10^{-11}	7	133 ± 43	101 ± 9	102 ± 11
(7–36)amide	10^{-10}	25	177 ± 23[c]	102 ± 10	100 ± 3
	10^{-9}	15	172 ± 33[a]	119 ± 8[a]	122 ± 10[a]
	10^{-8}	16	165 ± 22[c]	168 ± 24[b]	120 ± 6[c]
	10^{-7}	11	160 ± 23[a]	123 ± 18	122 ± 7[b]
Insulin	10^{-10}	5	140 ± 33	102 ± 12	102 ± 12
	10^{-9}	14	187 ± 40[a]	206 ± 45[a]	128 ± 11[a]
	10^{-8}	13	137 ± 15[a]	171 ± 27[a]	121 ± 5[c]

n, Number of experiments. [a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.01$; [d] $p < 0.001$

(–36)amide (10^{-10} mol/l) was pre-treated with a C-terminal GLP-1 antiserum, the effect on glycogen synthesis represented 56 ± 4 % (n = 7, $p < 0.001$) of that obtained in the paired muscle incubated with the untreated peptide.

GLP-1(7–36)amide, at 10^{-10} mol/l, significantly increased synthase *a* activity (126 ± 9 % of control paired value, n = 14, $p < 0.02$), while it did not significantly modify the phosphorylase *a* control value (95 ± 5 %, n = 14); at 10^{-9} mol/l, the corresponding mean values were similar to that at 10^{-10} mol/l (123 ± 11 % and 104 ± 9 %, n = 6), and not different from those obtained with 10^{-8} mol/l insulin (119 ± 7 % and 93 ± 10 %, respectively, n = 11).

Table 1 shows the glucose utilization, measured as ^{3}HOH formation from D-[5-^{3}H]glucose and as lactate release, and the glucose oxidation, determined as $^{14}CO_2$ production from D-[U-^{14}C]glucose. GLP-1(7–36)amide significantly increased glucose utilization and oxidation ($p < 0.001$, df = 147, by analysis of variance, at all parameters); at 10^{-10} mol/l, only the increment in glucose oxidation was statistically significant ($p < 0.01$). The three parameters were also significantly increased by insulin ($p < 0.01$, df = 63, by analysis of variance).

Cyclic AMP content was neither modified by 10^{-10} and 10^{-9} mol/l GLP-1(7–36)amide (100 ± 5 % of control, n = 12, and 102 ± 10 %, n = 8, respectively), nor by insulin at 10^{-8} mol/l (109 ± 10 %, n = 8).

Discussion

These data indicate that GLP-1(7–36)amide can directly stimulate glycogen synthesis in skeletal muscle. As is known for insulin [3], this stimulation is associated with an increase in the glycogen-synthase *a* activity and no change in glycogen phosphorylase *a* activity. GLP-1(7–36)amide exerted its maximal effect on glycogen synthesis at 10^{-10} mol/l, a concentration which has been documented as physiological [see Ref. 1, for review]; this effect was abolished when the peptide was linked to a C-terminal GLP-1 antibody; at the same concentration, it also maximally stimulated glucose oxidation. In addition, at higher concentrations of the peptide, it increased the rate of glucose utilization (^{3}HOH production from D-[5-^{3}H]glucose) and lactate formation, which is an index of glycolysis in this tissue.

These insulin-like effects of GLP-1(7–36)amide on glucose metabolism in skeletal muscle could be mediated by specific receptors [8, 9], but apparently not a G-protein-linked type such as that found in the pancreatic beta cell [10], since the GLP-1(7–36)amide did not increase the muscle cyclic AMP content.

Although the ultimate mechanism of this glycogenic action has not been elucidated, the data presented here suggest that skeletal muscle is one of the target tissues for GLP-1(7–36)amide, where this insulin-like effect explains, at least in part, the plasma glucose-lowering action of the peptide [2]; thus, GLP-1(7–36)amide may well be implicated in the physiological control of glucose homeostasis following meals, not only by acting as an incretin, but also by directly promoting glucose disposal.

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