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# Inositolphosphoglycans Possibly Mediate the Effects of Glucagon-Like Peptide-1(7-36)amide on Rat Liver and Adipose Tissue

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Insulin-like effects of glucagon-like peptide-1(7-36)amide (GLP-1) in rat liver, skeletal muscle and fat, and also the presence of GLP-1 receptors in these extrapancreatic tissues, have been documented. In skeletal muscle and liver, the action of GLP-1 is not associated with an activation of adenylate cyclase, and in cultured murine myocytes and hepatoma cell lines, it was found that GLP-1 provokes the generation of inositolphosphoglycan molecules (IPGs), which are considered second messengers of insulin action. In the present work, we document in isolated normal rat adipocytes and hepatocytes that GLP-1 exerts a rapid decrease of the radiolabelled glycosylphosphatidylinositols (GPIs) — precursors of IPGs — in the same manner as insulin, indicating their hydrolysis and the immediate short-lived generation of IPGs. Thus, IPGs could be mediators in the GLP-1 actions in adipose tissue and liver, as well as in skeletal muscle, through GLP-1 receptors which are, at least functionally, different from that of the pancreatic B-cell. © 1998 John Wiley & Sons, Ltd.

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KEY WORDS — GLP-1; inositolphosphoglycans (IPGs); glycosylphosphatidylinositols (GPIs); hepatocytes; adipocytes

## INTRODUCTION

Glucagon-like peptide-1(7-36)amide (GLP-1) is a glucose-dependent insulinotropic intestinal peptide released mainly after glucose ingestion,[1] with antidiabetogenic properties[2–4] and insulin-like effects upon glucose metabolism in rat extrapancreatic tissues such as liver,[5] skeletal muscle[6] and fat.[7,8] The presence of GLP-1 receptors in these tissues has been documented[9–15] and they seem to differ, in the signalling pathway, from that of the pancreatic B-cell[16] as, at least in liver and skeletal muscle, GLP-1 does not increase adenylate cyclase activity.[10,14] The available data on adipocytes are scanty and apparently contradictory.[17,18]

Insulin induces in liver,[19–21] skeletal muscle[22,23] and fat[24] cells, the generation of inositolphosphoglycans (IPGs), which are derived from membrane glycosylphosphatidylinositols (GPIs), by the action of a phosphatidylinositol-specific phospholipase C. IPGs mimic some of the effects of insulin in these extrapancreatic cells, and they are considered mediators of the insulin action.[25]

In BC3H-1 myocytes[26] and in HEP-G2 hepatoma[27] cell lines, not only the presence of [$^{125}$I] GLP-1 specific binding was found but also a stimulatory effect was demonstrated of GLP-1 upon glycogen synthesis and a modulation of the GPIs by the peptide, comparable to that observed with insulin within the same experiment.

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In this work, we report the effect of GLP-1, compared with that of insulin, on GPIs metabolism in isolated rat adipocytes and hepatocytes.

## MATERIALS AND METHODS

### *Materials*

Synthetic glucagon-like peptide-1(7-36)amide (Peninsula Lab. Inc., Belmont, CA, U.S.A.); rat insulin (Novo Biolabs, Bagsvaerd, Denmark); Fraction V bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, MO, U.S.A.); collagenase P and A (Boehringer Mannheim, Mannheim, Germany); phosphatidyl [2-$^3$H] inositol-4 monophosphate (PIP), L-3-phosphatidyl [2-$^3$H] inositol (PI) and *myo*-[$^3$H]inositol (Amersham International, Aylesbury, Buckinghamshire, U.K.); Ultima Gold scintillation liquid (Packard, Groningen, The Netherlands).

### *Cells*

Adipocytes and hepatocytes were isolated from the epididymal fat and the liver of male Wistar rats (150–200 g), by collagenase P or A enzymic digestion, respectively, as previously described.[28,29]

### *Labelling and Measurement of Glycosylphosphatidylinositols*

Adipocytes ($60 \times 10^6$ cells) were preincubated for 30 min at 37°C, in 4 ml Krebs–Ringer–Bicarbonate (KRB) containing 3 per cent BSA, 3·3 mM D-glucose, 500 kIU ml$^{-1}$ Trasylol and 10·9 mM HEPES, and then radiolabelled during 120 min with *myo*-[$^3$H]inositol (10 μCi ml$^{-1}$). Hepatocytes ($75 \times 10^6$ cells) were preincubated for 30 min at 37°C, in 10 ml 1·3 mM $Ca^{2+}$ modified KRB containing 2 per cent BSA and 15 mM D-glucose, and then radiolabelled during 60 min with *myo*-[$^3$H]inositol (15 μCi ml$^{-1}$). During the preincubation and incubation periods the cells were continuously gassed with 95 per cent $O_2$–5 per cent $CO_2$. Afterwards, both cell types were washed three times with their corresponding preincubation medium containing, in addition, 1 mM inositol. Radiolabelled adipocytes ($10^6$ cells per 300 μl) and hepatocytes ($10^6$ cells per 200 μl) were incubated for different time periods (0–10 min) at 37°C, in the same fresh corresponding medium, and in the absence or presence of $10^{-9}$ M GLP-1 or insulin. At the end of the incubation, and after the medium had been removed and discarded, the cellular GPIs were extracted following a procedure based on a previous published protocol:[30] in brief, cells were treated with 10 per cent trichloroacetic acid for 10 min at 4°C, and the precipitate, sedimented by centrifugation, was treated overnight at −20°C with 1 ml $CHCl_3/CH_3OH$/37 per cent HCl (1:2:0.0125); then 250 μl $CHCl_3$ and 250 μl 0·1 M KCl were added to form a two-phase system, the organic one being speed-vac dried and redissolved in 50 μl $CHCl_3/CH_3OH$ (2:1). The lipidic extract was applied to a silica gel thin-layer-chromatography (TLC) plate, and developed two consecutive times in $CHCl_3/CH_3COCH_3/CH_3OH/CH_3COOH/H_2O$ (50:20:10:10:5); a region taken from 2 cm above to 1 cm below the origin — where GPIs remain — was scraped and eluted with 2 ml methanol during 10 min at 37°C. After centrifugation, the supernatant was speed-vac dried, reconstituted in 50 μl $CHCl_3/CH_3OH$ (2:1), and applied to a TLC plate which was developed in $CHCl_3/CH_3OH$/25 per cent $NH_4OH/H_2O$ (45:45:4:10). In parallel, samples of radioactive PIP and PI, as migration markers, were also applied. The GPIs were detected by autoradiography, and their gel-scrapes were $\beta$-counted.

### *Statistical Evaluations*

Data are presented as mean ± SE, together with the number of experimental samples ($n$). The statistical significances were estimated by the Student $t$-test.

## RESULTS

### *Adipocyte GPIs*

The effect of $10^{-9}$ M GLP-1 on the GPIs content in isolated rat adipocytes, and that of $10^{-9}$ M insulin, is shown in Figure 1. GLP-1 induced a rapid decrease of the radiolabelled cellular GPIs content, detectable within 0·5 min incubation in the presence of the peptide, and maintained significantly lower for up to 1 min (0·5 min: 76 ± 7 per cent of control cells, incubated in the absence of peptide, $n = 7$, $p < 0{\cdot}02$; 1 min: 76 ± 5 per cent, $n = 8$, $p < 0{\cdot}01$). This decrease was followed by a progressive increase of the mean values which, after 2 and up to 10 min incubation, were significantly higher than that of the control (5 min: 138 ± 7 per cent, $n = 5$, $p < 0{\cdot}001$; 10 min: 128 ± 7 per cent, $n = 6$, $p < 0{\cdot}02$). With insulin, the same pattern of immediate decrease in the adipocyte radioactive

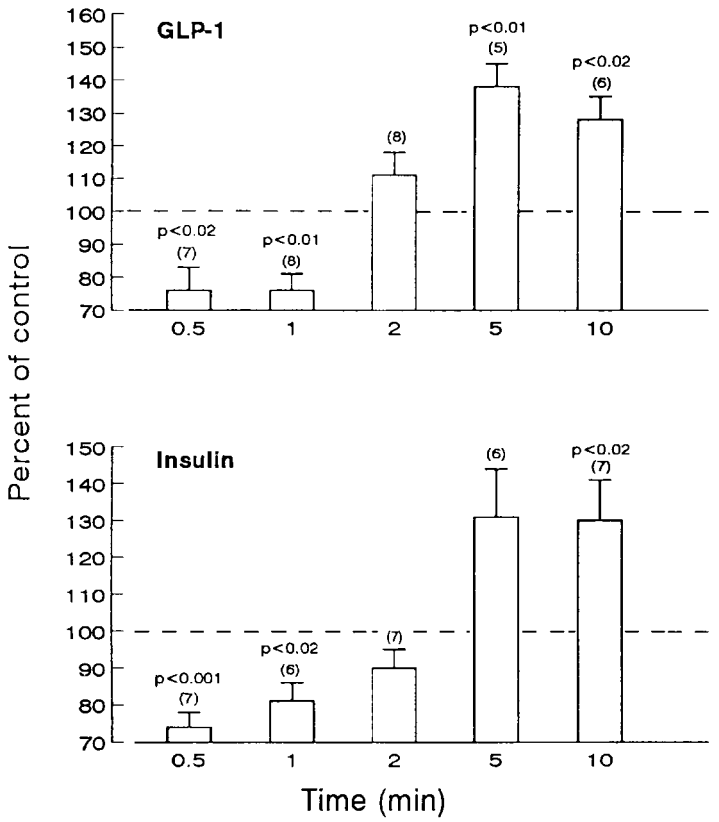


Figure 1. Time course of the effect of $10^{-9}$ M GLP-1 and $10^{-9}$ M insulin on the radioactive GPIs content in isolated rat adipocytes, prelabelled with *myo*-[$^3$H]inositol. Data are expressed as a percentage of the control value obtained in cells incubated in the absence of the peptide, and correspond to 5–6 individual experiments for each peptide.

GPIs content was observed (0·5 min: $74 \pm 4$ per cent of control, $n = 7$, $p < 0{\cdot}001$; 1 min: $81 \pm 5$ per cent, $n = 6$, $p < 0{\cdot}02$). After 1 min and up to 10 min, a progressive increase was also detected, reaching a value at 10 min that was significantly higher than that of the control (5 min: $131 \pm 13$ per cent, $n = 6$; 10 min: $130 \pm 11$ per cent, $n = 7$, $p < 0{\cdot}02$).

### *Hepatocyte GPIs*

The effects of $10^{-9}$ M GLP-1 and $10^{-9}$ M insulin on the GPIs content in isolated rat hepatocytes are shown in Figure 2. GLP-1 induced a rapid decrease of the radiolabelled cellular GPIs content, observed within 0·5 min incubation in the presence of the peptide, and remained significantly lower for up to 1 min (0·5 min: $78 \pm 3$ per cent of the control cells, incubated in the absence of peptide, $n = 15$, $p < 0{\cdot}001$; 1 min: $87 \pm 5$ per cent, $n = 14$, $p < 0{\cdot}05$). This decrease was followed by a progressive increase of the mean values which were, after 2 and up to 10 min incubation, significantly higher than that of the control (5 min: $119 \pm 6$ per cent, $n = 17$, $p < 0{\cdot}01$; 10 min: $112 \pm 5$ per cent, $n = 16$, $p < 0{\cdot}05$). With insulin, the same pattern of immediate decrease in the hepatocyte radioactive GPIs content was observed (0·5 min: $77 \pm 5$ per cent of control, $n = 17$, $p < 0{\cdot}001$; 1 min: $75 \pm 7$ per cent, $n = 12$, $p < 0{\cdot}01$) followed after 1 min and up to 10 min, by a progressive increase. The value at 10 min was significantly higher than that of the control (5 min: $113 \pm 5$ per cent, $n = 16$, $p < 0{\cdot}05$; 10 min: $115 \pm 4$ per cent, $n = 15$, $p < 0{\cdot}01$).

## DISCUSSION

Our data document that GLP-1 exerts an immediate and short-lived generation of IPGs in rat hepatocytes and adipocytes, similar to the effect of insulin, as shown by a decrease of the radioactive GPIs content during the first minute of incubation in the presence of the hormones. The increase of the radioactive GPIs after their hydrolysis is believed to be due to *de novo* synthesis

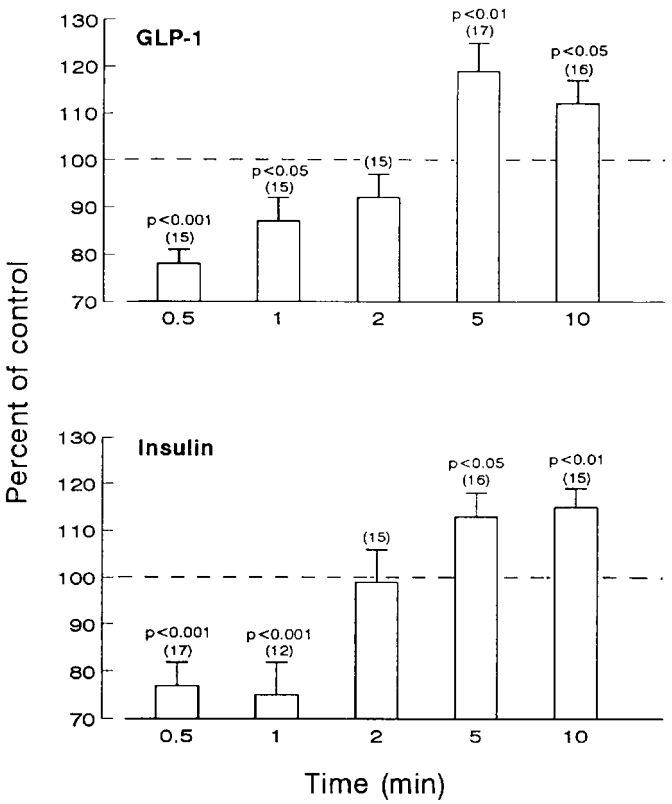


Figure 2. Time course of the effect of $10^{-9}$ M GLP-1 and $10^{-9}$ M insulin on the radioactive GPIs content in isolated rat hepatocytes, prelabelled with *myo*-[$^3$H]inositol. Data are expressed as a percentage of the control value obtained in cells incubated in the absence of the peptide, and correspond to 10 individual experiments for each peptide.

of phosphatidylinositol and its subsequent rapid conversion into GPIs.[22] We have previously observed this GLP-1 action in human hepatoma HEP-G2 cell line[26] and in murine BC3H-1 myocytes,[25] by using radioactive galactose as a marker of the precursor GPIs. In the present experiments, we have used radioactive *myo*-inositol, which also labels the precursor of one type of IPG molecule considered to be involved in the insulin action.[19,24]

The present data extend to normal rat hepatocytes the knowledge that these putative insulin mediators, IPGs, may also participate in the mechanism of action of the insulinomimetic effects of GLP-1 observed in rat liver.[5] [$^{125}$I]GLP-1 specific binding to rat hepatic membranes, not inhibited by insulin, has been documented,[14] and the presence of pancreatic GLP-1 receptor mRNA transcripts in rat[11,15] and mouse liver,[9] have been detected in apparent minor quantities than in pancreatic tissue; but the exact nature of the liver GLP-1 receptor is still unknown. The facts that the *N*-terminal extended GLP-1 (glucagon-like peptide-1(1-36)amide) inhibits [$^{125}$I]GLP-1 binding in the liver, with close to the same affinity as the unlabelled GLP-1, and that it exerts a glycogenic effect on rat hepatocytes with the same potency as GLP-1,[5] suggest that the GLP-1 receptor in this organ is not exactly the same as that in pancreas. Not only does the pancreatic receptor have a very low, if any, affinity for the *N*-terminal extended GLP-1,[31,32] but is also a poor insulin secretagogue.[33,34] Furthermore, GLP-1 does not modify adenylate cyclase activity in rat liver plasma membranes,[14] and it inhibits the cAMP content in rat hepatocytes when incubated in the absence of cAMP-specific phosphodiesterase inhibitors.[5] These effects are opposite to the action of GLP-1 on the pancreatic B-cell through its G-protein linked receptor.[16]

In adipose tissue, GLP-1 may have a dual effect on lipid metabolism, in that it can be lipolytic[18] and lipogenic.[7,8] Also, GLP-1 was shown to stimulate basal and/or insulin-induced glucose transport in rat adipocytes[8,17] and 3T3-L1 cells,[35]

and to increase glycogen synthesis in rat fat pieces.[8] By [$^{125}$I]GLP-1 binding studies, specific GLP-1 receptors were found in solubilized membranes of rat[13] and human adipose tissue,[12] and by PCR and Southern blot analysis, using the pancreatic GLP-1 receptor cDNA probe, the presence of mRNA for this receptor in rat epididymal fat and 3T3-L1 adipocytes was detected.[11] The exact structure of the GLP-1 receptors in this tissue, and in liver, remain to be elucidated, however. The present results indicate that a GLP-1 receptor, functionally distinct from the pancreatic one, is also present in adipose tissue, as it is in the liver and in skeletal muscle.[10]

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