Glucagon-like peptide-1(7–36)-amide confers glucose sensitivity to previously glucose-incompetent β-cells in diabetic rats: in vivo and in vitro studies

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Abstract

The effects of glucagon-like peptide-1(7–36)-amide (GLP-1) on cAMP content and insulin release were studied in islets isolated from diabetic rats (n0-STZ model) which exhibited impaired glucose-induced insulin release. We first examined the possibility of re-activating the insulin response to glucose in the β-cells of the diabetic rats using GLP-1 in vitro. In static incubation experiments, GLP-1 amplified cAMP accumulation (by 170%) and glucose-induced insulin release (by 140%) in the diabetic islets to the same extent as in control islets. Using a perifusion procedure, GLP-1 amplified the insulin response to 16·7 m\text{M} glucose by diabetic islets and generated a clear biphasic pattern of insulin release. The incremental insulin response to glucose in the presence of GLP-1, although lower than corresponding control values (1·56 ± 0·37 and 4·53 ± 0·60 pg/min per ng islet DNA in diabetic and control islets respectively), became similar to that of control islets exposed to 16·7 m\text{M} glucose alone (1·09 ± 0·15 pg/min per ng islet DNA). Since in vitro GLP-1 was found to exert positive effects on the glucose competence of the residual β-cells in the n0-STZ model, we investigated the therapeutic effect of in vivo GLP-1 administration on glucose tolerance and glucose-induced insulin release by n0-STZ rats. An infusion of GLP-1 (10 ng/min per kg; i.v.) in n0-STZ rats enhanced significantly (P<0·01) basal plasma insulin levels, and, when combined with an i.v. glucose tolerance and insulin secretion test, it was found to improve (P<0·05) glucose tolerance and the insulinogenic index, as compared with the respective values of these parameters before GLP-1 treatment.

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Introduction

Glucagon-like–peptide-1(7–37) or its amidated form, GLP-1(7–36)–amide (GLP-1), are products of post-translational processing of proglucagon in the mammalian intestine (Bell et al. 1983, Mojsov et al. 1986). GLP-1 is released into the circulation in response to an oral glucose load and potentiates glucose-stimulated insulin release (Göke et al. 1991). It is therefore considered to be an incretin important for the control of glucose homeostasis. The pancreatic β-cell contains specific receptors for GLP-1 (Göke & Conlon 1988). GLP-1 exerts diverse insulinotropic actions on β-cells including stimulation of cAMP formation (Drucker et al. 1987), insulin secretion (Schmidt et al. 1985, Mojsov et al. 1987), insulin biosynthesis and proinsulin gene expression (Fehmann & Habener 1992). Importantly, the stimulatory action of GLP-1 on insulin secretion requires the presence of glucose at normal or slightly elevated concentration (Drucker et al. 1987, Thorens & Waeber 1993). Using islets from rats with non-insulin-dependent diabetes induced by neonatal streptozotocin administration (n0-STZ), we have previously shown that n0-STZ β-cells exhibit impaired glucose-induced insulin release (Portha et al. 1988). We have also previously identified defective glucose-induced cAMP generation in their β-cells, but this defect was related to the glucose only and could mostly be explained by a block in the step(s) linking glucose metabolism and activation of adenylate cyclase without any major disturbance of adenylate cyclase per se (Dachicourt et al. 1996). The aims of the present study were to investigate in n0-STZ rats: (1) the effect of GLP-1 in vitro on cAMP accumulation and insulin release in isolated islets; (2) the possibility of re-activating in vitro the insulin response to glucose using GLP-1; (3) the therapeutic effect of GLP-1 administration on glucose tolerance and insulin secretion in vivo.
Materials and Methods

Animals
Albino Wistar rats bred in our laboratory were fed *ad libitum* with commercial pelleted chow (diet 113; Usine d’Alimentation Rationnelle, Villemoisson-sur-Orge, France). On the day of birth, rats received streptozotocin (100 µg/g, i.v.) diluted in 25 µl citrate buffer (0.05 mol/l, pH 4.5) as previously described (Porhafa et al. 1974). These rats were named n0-STZ. Non-diabetic Wistar rats were used as control animals. All animals used in the experiments were adult male rats (12–13 weeks old).

In vivo GLP-1 treatment
At 3 months of age a group of n0-STZ rats received a GLP-1 infusion (Sigma, St Louis, MO, USA) (10 ng/min per kg body weight) for 45 min while in the postabsorptive state and under pentobarbital anaesthesia (6 mg/100 g body weight). At 15 min after the beginning of the infusion, an i.v. glucose tolerance and insulin secretion test (IVGTT: 0.5 g/kg body weight) was performed. The diabetic rats had previously been tested 5 days before the GLP-1 infusion (IVGTT during saline infusion) and could therefore be used as their own controls. Blood samples were collected sequentially from the tail vein before and 5, 10, 15, 20 and 30 min after the injection of glucose (Merck, Darmstadt, Germany). They were then centrifuged and the plasma was separated. Plasma glucose concentration was immediately determined on a 10 µl aliquot and the remainder was kept at −20 °C until insulin RIA.

Islet isolation
Islets were isolated from the pancreases of two or four rats by collagenase (Boehringer, Mannheim, Germany) digestion (Lacy & Kostianovsky 1967) and subsequently separated from the remaining exocrine tissue by hand picking under a stereomicroscope. The islets were immediately used for static incubation or perifusion experiments.

Evaluation of islet cAMP content and insulin release during static incubation
Islet cAMP content and insulin release were measured during static incubation on the same batches of islets, in the islet pellets and the supernatant respectively, using RIAs. Briefly, after a preincubation period, groups of 20 islets were incubated for 15 min at 37 °C in 1.0 ml KRB buffer (Malaisse-Lagae & Malaisse 1984) supplemented with 5 mg/ml BSA (fraction V, Sigma, Deisenhofen, Germany) containing 8.3 mM glucose and/or 100 nM GLP-1. At the end of this period, the medium was stored at −20 °C until assayed for insulin using the RIA described below. Islet cAMP content was measured at the end of the incubation period as previously described (Dachicourt et al. 1996).

Evaluation of insulin release during perifusion experiments
Kinetics of insulin release in vitro were studied using the perifusion procedure described elsewhere (Dachicourt et al. 1996). Briefly, on the day of the experiment, 100 freshly isolated islets were placed in a column of perifusion medium (KRB buffer supplemented with 5 mg/ml BSA) at 37 °C. The perifusion medium was maintained at 37 °C and constantly gassed with a mixture of O2/CO2 (95%:5%). n-glucose and/or 100 nM GLP-1 were added to the perifusion medium as required. The perifusion fluid (1 ml/min) was collected in centrifuge tubes at 1 min intervals and frozen for storage at −20 °C until assayed for insulin.

Analytical techniques, data presentation and statistical analysis
Plasma glucose concentration was measured by the glucose oxidase method in 10 µl plasma using a glucose analyser (Beckman Instruments, Fullerton, CA, USA). Plasma insulin concentration was determined by RIA using purified rat insulin as standard (Novo, Copenhagen, Denmark), antibodies to mixed (pork and beef) insulin, and pork [125I]monoiiodinated insulin as tracer (Freychet et al. 1971). Charcoal was used to separate free from bound hormone. The method allowed the determination of 0.25 ng/ml, with a coefficient of variation within and between assays of 10%. DNA content in islets from control and n0-STZ rats was determined as previously described (Giroix et al. 1993). In the static incubation experiments, insulin release was expressed as either an absolute value (ng/15 min per ng islet DNA) or as the percentage of the basal release in each group of animals. cAMP accumulation was expressed as either an absolute value (fmol/15 min per ng islet DNA) or as the percentage of the basal cAMP accumulation. Insulin secretion rate during the perifusion experiments was calculated by multiplying the insulin concentration in the samples by the flow rate, and is expressed as pg/min per ng islet DNA. Total insulin response to glucose or GLP-1 was obtained by planimetry of the individual perifusion profiles and expressed as the difference in insulin secretion rate (pg insulin/min per ng islet DNA) from the mean hormonal output response recorded at the end of the prestimulation period. The insulin and glucose responses during the IVGTT were calculated as the incremental plasma insulin values integrated over a period of 30 min after the injection of glucose (ΔI) and the corresponding increase in glucose concentration (ΔG). The insulogenic index (ΔI/ΔG) represents the ratio of these two parameters.
All the results are presented as means ± s.e.m., and statistical significance of differences between means values was evaluated by Student’s t-test for unpaired data except for IVGTT experiments where Student’s t-test was assessed for paired data.

**Results**

**Biological characteristics of the non-diabetic controls and the diabetic n0-STZ rats**

The characteristics of normal Wistar (control) and diabetic n0-STZ rats used in the present study are shown in Table 1. The diabetic rats were the same age and had the same body weight as the control rats. When examined in the fed state, they exhibited a higher plasma glucose concentration (P<0.001) and a lower (P<0.05) plasma insulin concentration than the controls. The mean islet DNA content of islets from diabetic n0-STZ rats was only 61% (P<0.001) of that of controls. Results in this study were therefore calculated on the basis of islet DNA content.

**Regulation of islet cAMP content and insulin release by 100 nm GLP-1 in the diabetic n0-STZ rats**

Islet cAMP content and insulin release were measured after static incubation in the islet pellet and supernatant respectively, using the same islets (Fig. 1). The effect of 100 nm GLP-1 on cAMP accumulation and insulin release was studied at 8.3 mm glucose.

We have previously studied cAMP content and insulin release in n0-STZ rats in response to different glucose concentrations, and demonstrated that exposure to glucose concentrations that induced a rise in cAMP content in the control islets did not elicit any significant increase in cAMP in diabetic islets. This coincided with very low glucose-induced insulin release by the islets of diabetic rats (Dachicourt et al. 1996).

GLP-1 induced a significant increase in both cAMP (P<0.02) and insulin release (P<0.001) in pancreatic islets isolated from control rats. cAMP content was 0.55 ± 0.01 and 0.90 ± 0.07 fmol/15 min per ng islet DNA in response to 8.3 mm glucose alone and 8.3 mm glucose+100 nm GLP-1 respectively. When these results are expressed as a percentage of the basal value, GLP-1 is found to augment the cAMP content significantly (P<0.001) by (169%). Insulin release was 0.87 ± 0.03 and 1.15 ± 0.06 ng/15 min per ng islet DNA in response to 8.3 mm glucose alone and 8.3 mm glucose+GLP-1 respectively. When these results are expressed as a percentage of the basal value, GLP-1 is found to increase insulin release significantly (P<0.001) (by 140%) in control islets.

GLP-1 also induced a significant increase in both cAMP content (P<0.001) and insulin release (P<0.001) in diabetic islets. cAMP content was 0.45 ± 0.02 and 0.82 ± 0.08 fmol/15 min per ng islet in response to 8.3 mm glucose and 8.3 mm glucose+GLP-1 respectively. When the results are expressed as a percentage of the basal value, GLP-1 is shown to increase the cAMP content (by 187%, P<0.001) to the same extent as in control rats. Insulin release was 0.68 ± 0.03 ng/15 min per ng islet DNA in response to 8.3 mm glucose and 0.96 ± 0.06 ng/15 min per ng islet DNA in response to 8.3 mm glucose+GLP-1. GLP-1 therefore augmented insulin release (by 148%, P<0.001) to the same extent as in control rats.

**In vitro kinetics of the glucose-induced insulin release in the absence or presence of GLP-1**

Kinetics of insulin release in vitro by islets of control and diabetic n0-STZ rats were tested using the perfusion procedure. The basal rate of insulin release measured at 2.8 mm glucose was significantly decreased (P<0.001) in diabetic rats (0.24 ± 0.009 pg/min per ng islet DNA) compared with control rats (0.29 ± 0.007 pg/min per ng islet DNA) (Fig. 2). After exposure to 16.7 mm glucose, a biphasic pattern of insulin release was observed in the control islets (Fig. 2, left panel). The first phase peaked 6–7 min after the change from low (2.8 mm) to high (16.7 mm) glucose. The rate of insulin secretion in the second phase reached a plateau approximately 20 min after the switch. Incremental insulin response to glucose for the

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**Table 1** Biological characteristics of non-diabetic control and diabetic n0-STZ rats. Values are means ± s.e.m. for the number of animals (in the fed state) or batches of islets shown in parentheses.

<table>
<thead>
<tr>
<th>Rats</th>
<th>Age (weeks)</th>
<th>Body weight (g)</th>
<th>Plasma glucose concentration (mmol/l)</th>
<th>Plasma insulin concentration (ng/ml)</th>
<th>Islet DNA content (ng/islet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>12.8 ± 0.7</td>
<td>357.7 ± 11.9</td>
<td>6.6 ± 0.2 (27)</td>
<td>2.5 ± 0.2 (27)</td>
<td>21.9 ± 0.9 (53)</td>
</tr>
<tr>
<td>n0-STZ</td>
<td>12.9 ± 0.4</td>
<td>334.2 ± 9.2</td>
<td>8.6 ± 0.3** (31)</td>
<td>2.1 ± 0.1* (31)</td>
<td>13.3 ± 0.6** (42)</td>
</tr>
</tbody>
</table>

Islet DNA content was determined in batches of islets (20 islets each) collected from 20–25 distinct islet preparations, each of them being obtained from 2–3 animals.

*P<0.05, **P<0.001 compared with control rats.
first and second phases of insulin secretion were respectively 0.39 ± 0.12 and 0.64 ± 0.14 pg/min per ng islet DNA. After suppression of the stimulus, insulin output decreased rapidly. In the diabetic rats exposure to 16.7 mM glucose induced only a very modest increase in insulin output (Fig. 2, right panel). The incremental insulin response to 16.7 mM glucose was 4.4-fold decreased ($P < 0.001$) in diabetic rats compared with control rats (Table 2, line 2).

In perifused control islets the addition of 100 nM GLP-1 to the perfusion medium amplified the secretory response to 16.7 mM glucose 4.2-fold ($P < 0.001$) (Table 2, lines 2 and 3). Indeed, in control rats, GLP-1 potentiated both the first and the second phases of glucose-induced insulin secretion (respectively 1.19 ± 0.20 and 2.72 ± 0.37 pg/min per ng islet DNA). It is important to notice that GLP-1 at low glucose concentration (2.8 mM) was without significant effect on insulin release (Fig. 2, left panel). In the diabetic rats, GLP-1 re-activated the insulin response to 16.7 mM glucose (6.3-fold) and restored the biphasic pattern of insulin release (Fig. 2, right panel). The incremental insulin response to glucose in the presence of GLP-1, although lower than corresponding control values (1.56 ± 0.37 and 4.53 ± 0.60 pg/min per ng islet DNA in diabetic and control rats respectively), became similar to that of control islets exposed to 16.7 mM glucose alone (1.09 ± 0.15 pg/min per ng islet DNA). In diabetic rats, the incremental insulin response to glucose in the presence of GLP-1 estimated for the first and second phases of insulin secretion were respectively 0.79 ± 0.20 and 0.88 ± 0.25 pg/min per ng islet DNA. GLP-1 at low glucose concentration (2.8 mM) was
**Effects of in vivo GLP-1 treatment in the diabetic n0-STZ rats: glucose tolerance and insulin secretion after a 45 min GLP-1 infusion**

A group of n0-STZ rats received a GLP-1 infusion (10 ng/min per kg body weight) for 45 min. At 15 min after the beginning of the GLP-1 infusion, an IVGTT was performed in the postabsorptive state. In parallel, a group of diabetic rats tested under the same conditions but receiving an infusion of water did not show any difference in the parameters studied compared with results obtained before treatment (data not shown). The GLP-1 infusion did not affect basal plasma glucose in the n0-STZ rats (corresponding to the value of the glycaemia at time 0 min, just before glucose injection). At 5 min after the glucose load, plasma glucose concentration increased to the same extent in the two groups. At 10, 15, 20 and 30 min after the glucose load, plasma glucose concentrations were significantly lower in n0-STZ rats infused with GLP-1 than in untreated n0-STZ rats (Fig. 3). Therefore in the diabetic rats the GLP-1 infusion improved glucose tolerance and insulin secretion.

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**Table 2** Incremental insulin response (ΔI) to 2·8 mM glucose +100 nM GLP-1, 16·7 mM glucose and 16·7 mM glucose +100 nM GLP-1, by perfused islets from control and diabetic n0-STZ rats. Values are means ± s.e.m. for the number of perfusions shown in parentheses.

<table>
<thead>
<tr>
<th>Line</th>
<th>Secretagogues</th>
<th>Controls</th>
<th>n0-STZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2·8 mM glucose +100 nM GLP-1</td>
<td>0·06 ± 0·03 (5)</td>
<td>0·01 ± 0·004 (5)</td>
</tr>
<tr>
<td>2</td>
<td>16·7 mM glucose</td>
<td>1·09 ± 0·15 (13)</td>
<td>0·25 ± 0·07** (12)</td>
</tr>
<tr>
<td>3</td>
<td>16·7 mM glucose +100 nM GLP-1</td>
<td>4·53 ± 0·59 (5)</td>
<td>1·56 ± 0·37* (5)</td>
</tr>
</tbody>
</table>

The value of ΔI was obtained by planimetry of the individual perfusion profiles and expressed as the difference in insulin secretion rate (pg insulin/min per ng DNA islet) relative to the mean hormonal output response recorded at the end of the prestimulatory period.

*P<0·01, **P<0·001 compared with respective control rats.

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**Figure 2** Insulin secretory response to the combination of 2·8 mM glucose (G)+100 nM GLP-1 (■, ●), to 16·7 mM glucose (○, △) and to the combination of 16·7 mM glucose+100 nM GLP-1 (●, ▲) by perfused islets isolated from normal control (left panel, ■, ○, ●) and diabetic n0-STZ (right panel, ●, △, ▲) rats. Values are means ± s.e.m. of five or more individual observations in each group of rats.
tolerance significantly, as further indicated by determination of the glucose disappearance rate or $K_G$ ($P<0.01$). The GLP-1 infusion increased basal plasma insulin ($P<0.01$) in the n0-STZ rats, and the insulin response to the glucose load was significantly increased 5 and 15 min after the glucose load (Fig. 3). The improvement in glucose tolerance and insulin secretion during the GLP-1 infusion was further evidenced by the fact that the insulinogenic index ($\Delta I/\Delta G$) was significantly higher ($P<0.05$). The values of the $\Delta I/\Delta G$ index were 1.62 ± 0.26 and 1.01 ± 0.20 in the n0-STZ rats after and before GLP-1 treatment respectively.

Discussion

GLP-1 stimulates insulin secretion and increases cAMP content in normal islets. Our findings are in agreement with the available evidence that the insulinotropic effect of GLP-1 is secondary to an increase in the cellular cAMP level caused by the activation of adenylate cyclase coupled to the β-cell GLP-1 receptor (Thorens & Waeber 1993, Widmann et al. 1994, Ahrén 1996) and the subsequent activation of protein kinase A and phosphorylation of key proteins in the signalling pathway of insulin release.

The first aim of the present study was to investigate the effects of GLP-1 on cAMP content and insulin release in islets isolated from diabetic rats which exhibit impaired glucose-induced insulin release (Portha et al. 1988). Our results show that GLP-1 amplified cAMP accumulation and insulin secretion in response to glucose in diabetic islets to the same extent as in control islets. From these results it follows that adenylate cyclase in the islets of n0-STZ rats is responsive to activation by GLP-1. It also suggests that there is no major alteration in the functionality of the adenylate cyclase in the β-cells of the diabetic rats, a conclusion that confirms and extends those previously reported by us (Dachicourt et al. 1996). In the β-cells of n0-STZ rats, we have previously identified defective glucose-induced cAMP generation, but this defect was found to be related to the response to glucose only and could be mostly explained by a block in the step(s) linking glucose metabolism and activation.
of adenylate cyclase, without any major disturbance of adenylate cyclase \textit{per se} (Dachicourt et al. 1996).

Since it is known that \textit{in vitro} treatment of glucose-incompetent adult \(\beta\)-cells with GLP-1 confers glucose-sensitive insulin release (Holz et al. 1993) and that glucose-unresponsive human fetal \(\beta\)-cells can be rendered partially glucose competent by GLP-1 \textit{in vitro} (Otonkoski & Hayek 1995), the second purpose of this work was to examine the possibility of re-activating the insulin response to glucose in \(\beta\)-cells of diabetic rats using GLP-1 \textit{in vitro}. Our results show that the direct exposure of perfused islets of diabetic rats to GLP-1 amplified their insulin response to high glucose concentration and generated a clear biphasic pattern of insulin release. These observations are in accordance with our previous data obtained \textit{in vitro} with isobutylmethylxanthine, glucagon and gastric inhibitory peptide under the same experimental conditions (Dachicourt et al. 1996). Therefore it may be concluded that artificially raising the intracellular cAMP level in \(\beta\)-cells of n0-STZ rats restores their responsiveness to glucose. The loss of glucose-induced insulin release is therefore not irreversible in the n0-STZ rat model, and the \(\beta\)-cell incompetence in this model does not reflect a permanent lesion due to the toxic action of streptozotocin, as suggested previously (Weir et al. 1986, Grill et al. 1987, Eizirik et al. 1988, Inoué et al. 1994).

Since GLP-1 was found to exert positive effects \textit{in vitro} on the glucose competence of the residual \(\beta\)-cells in the n0-STZ model, the third aim of the present work was to investigate the therapeutic effect of GLP-1 administration \textit{in vivo} on glucose tolerance and insulin secretion in n0-STZ rats. The purpose of our \textit{in vivo} protocol was to assess the effects of acute administration of GLP-1. The effect of a 45 min GLP-1 infusion was investigated in anaesthetized rats. Our results show a clear improvement in the insulinogenic index \textit{in vivo} in the diabetic rats infused with GLP-1, which is directly related to the positive effect of GLP-1 upon the glucose competence of the \(\beta\)-cells in the n0-STZ model of diabetes. The GLP-1-induced enhancement of the insulin response to glucose \textit{in vivo} may explain the clear improvement in tolerance to glucose. Alternatively the possibility that this effect of GLP-1 had occurred, at least partly, via recognized extrapancreatic actions cannot be ruled out. Indeed GLP-1 has been reported to enhance insulin-stimulated glucose uptake by the peripheral tissues in the normal rat (Villanueva-Penacerrillo et al. 1994, Tominaga et al. 1996, Van Dijk et al. 1996) as well as in insulin-resistant OLET rat, a model of non-insulin-dependent diabetes mellitus (Mizumo & Shimma 1996), or alloxan diabetic mouse (Ahrén 1994), and to reduce the hepatic glucose delivery in normal rats (Van Dijk et al. 1996).

In conclusion, the present study demonstrates the usefulness of GLP-1 in acutely improving competence to glucose of the residual \(\beta\)-cells and the tolerance to glucose in the n0-STZ rat, a well recognized model of non-insulin-dependent diabetes mellitus.

Acknowledgements

Parts of this work were presented at the 31st Annual Meeting of the European Association for the Study of Diabetes, Stockholm, 12–16 September 1995, and have appeared in abstract form (Diabetologia (1995) 38: (Suppl 1) I-VI, 663:A171).

References


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