Effects of glucagon-like peptide-1-(7–36)-amide on pancreatic islet and intestinal blood perfusion in Wistar rats and diabetic GK rats

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ABSTRACT

The aim of the present study was to evaluate the effects of GLP-1 [glucagon-like peptide-1-(7–36)-amide] on total pancreatic, islet and intestinal blood perfusion in spontaneously hyperglycaemic GK rats and normal Wistar rats using a microsphere technique. GK rats had hyperglycaemia and increased pancreatic and islet blood flow. Blood glucose concentrations were not affected when measured shortly (8 min) after GLP-1 administration in either GK or Wistar rats. GLP-1 had no effects on baseline pancreatic or islet blood flow in Wistar rats, but did prevent the blood flow increase normally seen following glucose administration to these animals. In GK rats, administration of GLP-1 decreased both pancreatic and islet blood flow. Glucose administration to the GK rats decreased pancreatic and islet blood flow. This decrease was not affected by pre-treatment with GLP-1. We conclude that administration of GLP-1 leads to a decrease in the augmented blood flow seen in islets of diabetic GK rats. The GLP-1-induced action on islet blood perfusion may modulate output of islet hormones and contribute to the antidiabetogenic effects of the drug in Type 2 diabetes (non-insulin-dependent diabetes).

INTRODUCTION

Type 2 diabetes is characterized by impaired glucose-induced insulin release, which is accounted for by a defective stimulus–secretion coupling of glucose in the pancreatic β-cells [1,2]. GLP-1 (glucagon-like peptide-1) is derived through the post-translational processing of pre-proglucagon in L-cells of the lower intestine [3,4]. The peptide is secreted mainly as GLP-1-(7–36)-amide [hereafter referred to as GLP-1; [3]]. GLP-1 has been shown to be a potent glucose-dependent stimulator of insulin secretion in different systems, including diabetic rats and humans [5–9]. GLP-1 also controls post-prandial glucose concentrations in normoglycaemic rats and humans [10–12], as well as in patients with Type 2 diabetes [11,13]. In addition to its effects on insulin secretion, the actions of GLP-1 include suppression of glucagon secretion, stimulation of (pro)insulin biosynthesis, prolongation of the rate of gastric and intestinal emptying, reduction of gastric acid secretion and mediation of the ‘ileal brake’ function [3,14,15]. Other potentially beneficial effects in humans include regulation of satiety [16]. GLP-1 binds to a seven-transmembrane-domain receptor [17,18], which couples to several G-proteins [19]. GLP-1 binding activates p38 MAPK (mitogen-activated protein kinase) [19] as well as adenylyl cyclase, leading to increased generation of cAMP and, further downstream, modulation of several regulatory steps in glucose

Key words: GK rat, glucagon-like peptide-1-(7–36)-amide (GLP-1), islet blood flow, Type 2 diabetes (non-insulin-dependent diabetes).

Abbreviations: BP, blood pressure; GLP-1, glucagon-like peptide-1-(7–36)-amide.

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stimulus–secretion coupling in the β-cell [20–22]. GLP-1 receptors are present on α-, β- and δ-cells in the islets [23].

The described effects of GLP-1 on insulin and glucagon release and gastric emptying constitute a basis for a strong antidiabetic action of GLP-1 in patients with Type 2 diabetes [3,4,11]. However, the rapid degradation of GLP-1 in the circulation limits its therapeutic use, but the development of incretin mimetics have led to the use of these substances as an adjunctive therapy in Type 2 diabetes [24,25].

We have proposed previously [26,29] that changes in the pancreatic islet microcirculation could contribute to impeded insulin output in diabetic subjects. Islet blood flow is regulated independently from that to the whole gland, and augmented islet blood perfusion has been demonstrated in various models for glucose intolerance and diabetes, including obese animals and non-obese GK rats [26–28]. Furthermore, the long-term increase in islet blood perfusion with an associated rise in capillary BP (blood pressure) may lead to structural and functional damage to the islet blood vessels [29]. In view of GLP-1 analogues emerging as treatments for Type 2 diabetes, we have examined the effects of GLP-1 on total pancreatic, islet and intestinal blood flow.

MATERIALS AND METHODS

Animals

Female GK-Wistar (GK) rats, aged 3–4 months, were from a local breeding colony (Department of Endocrinology, Karolinska Hospital, Stockholm, Sweden), established in 1988 with breeding couples obtained from the colony at the Tohoku University School of Medicine. The GK strain originated from normal Wistar rats, which were bred using as selection criteria for mating high glucose values in an oral glucose tolerance test [30,31]. Age-matched control Wistar rats were purchased from B&K. All animals were kept under standard conditions with free access to tap water and pelleted food. The study was approved by the local Animal Ethics Committee at Uppsala University.

Blood flow measurements

The experiments were performed according to a protocol described in detail previously [32]. Briefly, non-fasted Wistar or GK rats were anaesthetized with an intra-peritoneal injection of sodium pentobarbital (50 mg/kg of body weight; Mebumal vet®; Apoteksbolaget), heparinized and placed on a heated operating table. Polyethylene catheters were inserted via the right carotid artery into the ascending aorta and into the femoral artery. The cranial catheter was connected to a pressure transducer (PDCR 75;1; Druck) thereby allowing constant monitoring of the mean arterial BP. After a stable BP was achieved, blood samples were drawn for measurement of the blood glucose concentration. GLP-1 (Sigma–Aldrich) dissolved in 0.2 ml of saline, or the vehicle alone, was then injected intravenously at a dose of 33 µg/kg of body weight. After 5 min, another intravenous injection consisting of 1 ml of saline or 30% (w/v) glucose was performed. These animals will be referred to as either saline-treated or glucose-treated below. At 3 min after the second injection, i.e. 8 min after the first injection, (1.5–2.0) × 10⁶ non-radioactive microspheres (NEN-Trac®; DuPont Pharmaceuticals) with a mean diameter of 11 µm were injected during 10 s via the catheter placed with its tip in the ascending aorta. Starting 5 s before microsphere injection and continuing for a total of 60 s, an arterial blood sample was collected from the catheter in the femoral artery at a rate of approx. 0.30 ml/min. The exact withdrawal rate was determined in each animal by weighing the sample. After obtaining the reference sample, another blood sample was drawn for measurement of blood glucose and serum insulin concentrations. After the animals were killed, the pancreas and adrenal glands as well as samples from the duodenum and colon were removed, blotted and weighed. The tissue samples were then treated with a freeze–thawing technique to visualize the microspheres as described previously [33]. Blood flow values were calculated according to the formula $Q_{org} = Q_{ret} \times \frac{N_{org}}{N_{ref}}$, where $Q_{org}$ is organ blood flow (in ml/min), $Q_{ret}$ is withdrawal rate of the reference sample (in ml/min), $N_{org}$ is number of microspheres present in the organ, and $N_{ref}$ is number of microspheres in the reference sample. A difference <10% in blood flow values between the adrenal glands was used to confirm adequate mixing of the spheres in the circulation.

Measurements of blood glucose and serum insulin concentrations

Arterial blood samples were obtained after securing the reference blood sample and were analysed later for blood glucose concentrations with a blood glucose meter (ExacTech®; Baxter Travenol). Serum insulin concentrations were determined by RIA (Pharmacia Insulin RIA-kit®, Pharmacia-Upjohn Diagnostics) with rat insulin (Novo Nordic) as a standard.

Statistical analysis

All values are means ± S.E.M. Data were compared using ANOVA with Bonferroni’s correction (Sigmasta®; SSPS Science Software).

RESULTS

Blood glucose concentrations

Saline-treated GK rats had higher blood glucose concentrations than corresponding Wistar rats ($P < 0.001$; Table 1). Administration of GLP-1 did not affect blood
Table 1  Effect on blood glucose and insulin concentrations of pre-treatment with GLP-1 prior to saline or glucose administration in female Wistar and GK rats

Female Wistar or GK rats were injected intravenously with 0.2 ml of saline or 10 nmol of GLP-1/kg of body weight (GLP) dissolved in 0.2 ml of saline at time 0 (substance 1). Blood glucose and insulin concentrations were measured. After 5 min, 1 ml of saline or a 30% solution of glucose was injected intravenously (substance 2). At 8 min after the injection of substance 1, blood glucose and insulin concentrations were measured again. Values are means ± S.E.M. for eight animals in each group. **P < 0.01 and ***P < 0.001 when compared with the corresponding saline-injected animals of the same strain. †P < 0.05 compared with saline + glucose-injected rats of the same strain. Note that all blood glucose concentrations in GK rats were significantly higher (P < 0.05 for all comparisons) when compared with the corresponding Wistar rats, whereas no differences in serum insulin concentrations can be seen.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rat strain</th>
<th>Substance 1</th>
<th>Substance 2</th>
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<tr>
<td></td>
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<td>Saline</td>
<td>GLP</td>
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<tr>
<td>Wistar rats</td>
<td>Blood glucose (mmol/l)</td>
<td>0 min</td>
<td>5.4 ± 0.3</td>
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<td></td>
<td>Serum insulin (ng/ml)</td>
<td>0 min</td>
<td>1.98 ± 0.20</td>
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<td></td>
<td>8 min</td>
<td>5.2 ± 0.2</td>
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<tr>
<td></td>
<td>Serum insulin (ng/ml)</td>
<td>8 min</td>
<td>2.32 ± 0.60</td>
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Glucose concentrations in saline-treated Wistar or GK rats. Glucose treatment led to a higher degree of hyperglycaemia in control GK rats compared with the corresponding Wistar rats (P < 0.001). GLP-1 pre-treatment of glucose-injected Wistar or GK rats did not affect blood glucose levels when compared with the corresponding saline-treated animals.

Serum insulin concentrations

There were no differences in serum insulin concentrations between saline-treated Wistar or GK rats (Table 1). GLP-1 did not affect the insulin concentrations in either group of animals. As expected, glucose injection increased insulin concentrations in all animals. GLP-1 potentiated the increase in insulin levels seen in both GK and Wistar rats following administration of glucose.

Total pancreatic and islet blood flow

Mean arterial BP was similar (110–120 mmHg) in all GK and Wistar rats and was unaffected by any of the treatments (results not shown). In saline-treated GK rats, both the whole pancreatic (Figure 1) and islet (Figure 2) blood flows were higher when compared with the corresponding Wistar rats. Administration of GLP-1 did not affect total pancreatic or islet blood flow in saline-treated Wistar rats. When the effect of GLP-1 was compared between saline-treated Wistar and GK rats, a decrease in both total pancreatic and islet blood flow was seen in GK rats.

Glucose-treated Wistar rats had higher total pancreatic and islet blood flow values than saline-treated controls. Glucose-treated GK rats, on the other hand, had a lowering of total pancreatic and islet blood flow when compared with both saline-treated GK rats and glucose-treated Wistar rats.

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Figure 2 Effect on pancreatic islet blood flow of pre-treatment with GLP-1 prior to saline or glucose administration in female Wistar and GK rats

Female Wistar (closed bars) and GK (grey bars) rats were pre-treated with an intravenous injection of 0.2 ml of saline (Sal) or 10 nmol of GLP-1/kg of body weight (GLP) 8 min before pancreatic islet blood flow measurements. After 5 min (i.e. 3 min before the blood flow measurements) an intravenous injection of 1 ml of saline (Saline) or a 30% D-glucose (Glucose) solution was given. Values are means ± S.E.M. for eight experiments. *P < 0.01 compared with the saline-pre-treated normoglycaemic Wistar or GK rats; **P < 0.01 compared with the corresponding group of Wistar rats.

Administration of GLP-1 to glucose-treated Wistar rats prevented the glucose-induced increase in total pancreatic and islet blood flow seen in these animals. When GLP-1 was given to glucose-treated GK rats, both total pancreatic and islet blood flow values remained low and did not differ from those of control glucose-treated GK rats. When compared with the glucose-treated GLP-1-injected Wistar rats, no differences were seen in either whole pancreatic or islet blood flow.

GLP induced similar changes in total pancreatic and islet blood flow in both Wistar and GK rats, that is the fraction of total pancreatic blood flow diverted through the islets remained constant (results not shown).

**Duodenal and colonic blood flow**

Duodenal (Figure 3), but not colonic (Figure 4), blood flow was increased in control GK rats compared with Wistar rats. Glucose treatment did not change duodenal blood flow in Wistar rats; however, in GK rats, administration of glucose decreased duodenal blood flow to values similar to those of saline-treated Wistar rats. Colonic blood flow was unaffected by glucose administration in both rat strains. Administration of GLP-1 had no effect on intestinal blood flow in either normoglycaemic Wistar rats. In saline-pre-treated GK rats, GLP-1 decreased both duodenal and colonic blood flow. There was no effect on intestinal blood flow by GLP-1 administration to glucose-pre-treated GK rats.

Figure 3 Effect on duodenal blood flow of pre-treatment with GLP-1 prior to saline or glucose administration in female Wistar and GK rats

Female Wistar (closed bars) and GK (grey bars) rats were pre-treated with an intravenous injection of 0.2 ml of saline (Sal) or 10 nmol of GLP-1/kg of body weight (GLP) 8 min before duodenal blood flow measurements. After 5 min (i.e. 3 min before the blood flow measurements) an intravenous injection of 1 ml of saline (Saline) or a 30% D-glucose (Glucose) solution was given. Values are means ± S.E.M. for eight experiments. *P < 0.01 compared with the saline-pre-treated normoglycaemic Wistar rats; **P < 0.01 compared with the corresponding group of Wistar rats.

Figure 4 Effect on colonic blood flow of pre-treatment with GLP-1 prior to saline or glucose administration in female Wistar and GK rats

Female Wistar (closed bars) and GK (grey bars) rats were pre-treated with an intravenous injection of 0.2 ml of saline (Sal) or 10 nmol of GLP-1/kg of body weight (GLP) 8 min before duodenal blood flow measurements. After 5 min (i.e. 3 min before the blood flow measurements) an intravenous injection of 1 ml of saline (Saline) or a 30% D-glucose (Glucose) solution was given. Values are means ± S.E.M. for eight experiments. *P < 0.01 compared with the saline-pre-treated normoglycaemic Wistar rats; **P < 0.01 compared with the corresponding group of Wistar rats.

**DISCUSSION**

In accordance with previous results obtained in both GK rats and mildly diabetic F1 hybrids of male GK and C57BL/6J mice.
female Wistar rats [26,27], basal pancreatic and islet blood flows were augmented in GK rats compared with control Wistar rats. Vagotomy has been shown to decrease the augmented islet blood flow in diabetic F1 hybrids [26] and GK rats [34] to values similar to those seen in vagotomized control rats. The same effect was achieved after treatment with atropine (L. Jansson, unpublished work), suggesting that a nervous cholinergic mechanism is, at least in part, responsible for the increased islet blood flow in the GK rat model. Later experiments have suggested that it is likely that ambient hyperglycaemia accounts for this nervous-system-mediated islet blood flow increase [35]. In the present study, we also confirmed our previous findings that glucose administration leads to an acute increase in islet blood flow in Wistar rats [29], whereas a decrease is seen in GK rats [26]. The glucose-induced islet blood flow increase in control rats is due to a complex interplay between nervous-system-mediated and metabolically induced signals [29,36]. The mechanisms underlying the glucose-induced decrease in islet blood flow in GK rats is unknown; however, nervous signalling pathways are likely to be involved [27].

Administration of GLP-1 had no effects on total pancreatic, islet, duodenal or colonic blood flow in normoglycaemic Wistar rats, suggesting that the hormone is not involved in the regulation of splanchnic blood flow during post-absorptive conditions. In view of the incretin effects and the alleged mediation of ileal brake functions of GLP-1 [3], i.e. important post-prandial functions, this is hardly surprising. In contrast, GLP-1 prevented the glucose-induced increase in total pancreatic and islet blood flow in hyperglycaemic Wistar rats. Serum insulin concentrations were increased in these animals, thereby confirming the incretin effect of the hormone. Regarding the finding of a marked reduction in the islet blood flow response to hyperglycaemia after GLP-1 administration in the Wistar rats, it may be speculated that binding of GLP-1 to specific receptors in the vasculature, and subsequent formation of cAMP, prevents a dilation of blood vessels. Indeed, specific GLP-1-binding sites were demonstrated in smooth muscle cells in the pulmonary artery of the rat [37]; however, administration of GLP-1 to pre-constricted isolated arterial rings with intact endothelium induced an atropin-resistant relaxation and no further constriction. Furthermore, receptors for GLP-1 in the pancreatic vasculature have not been reported to our knowledge. Alternatively, GLP-1 may release other gastrointestinal hormones, which then modulate islet blood flow. Somatostatin has been shown to decrease splanchnic blood flow [38–40], including islet blood flow [41], and the secretion of this peptide is increased by GLP-1 [42].

Of interest in this context is whether the prevention of glucose-induced islet blood flow increase is involved in the ileal brake mechanism mediated by GLP-1 and GLP-2 (glucagon-like peptide-2) with a general inhibition of upper gastrointestinal tract functions, as also manifested in, for example, delayed gastric emptying [3,43,44]. However, this notion remains conjectural. It should be noted that the other major incretin, GIP (gastric-inhibitory peptide), potentiates the glucose-induced increase in islet blood [45], suggesting different mechanisms of action in regulation of islet blood flow for these two peptides.

When GLP-1 was given to GK rats, both total pancreatic and islet blood flow were decreased to values similar to those seen in normoglycaemic Wistar rats. We chose to express islet blood flow per gram of pancreas, since we have observed previously [46] that there are no differences in islet volume between Wistar and GK rats at the ages used in the present study. In view of the potential therapeutic benefits of GLP-1 as an adjunct therapy in Type 2 diabetes, the islet-blood-flow-decreasing properties of GLP-1 may be of importance, since hyper-perfusion has been suggested to be harmful to several organs in the body, for example, in the development of diabetic vascular complications [47,48]. A reduction of increased basal levels of islet blood flow could therefore have beneficial effects on islet function in a long-term perspective (c.f. [38]). It should be noted that the GLP-1-induced decrease in blood flow does not adversely affect the possibility to secrete insulin, as evident from the potentiated insulin secretion in Wistar rats and the unchanged secretion in GK rats.

Of considerable interest were the findings that GLP-1 had no effects on duodenal or colonic blood flow in Wistar rats, whereas a significant decrease in both was seen in GK rats. The functional importance of this is unknown, but a speculative interpretation would be that GK rats have a hyper-reactive splanchnic vasculature.

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