Stimulation of intestinal glucoreceptors in rats increases pancreatic islet blood flow through vagal mechanisms

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Carlsson, Per-Ola, Masanori Iwase, and Leif Jansson. Stimulation of intestinal glucoreceptors in rats increases pancreatic islet blood flow through vagal mechanisms. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R233-R236, 1999.—The aim of the study was to evaluate whether intestinal glucoreceptors participate in the regulation of pancreatic islet blood flow. For this purpose, anesthetized rats were infused (0.1 ml/min for 3 min) with saline, glucose, or 3-O-methylglucose directly into the duodenum. The glucose (1 mg/kg body wt) infusion rate was chosen to prevent any effects on systemic or intraportal blood glucose concentrations. Intraduodenal infusion of d-glucose increased both serum insulin concentration and islet blood flow, whereas the osmotic control substance 3-O-methylglucose had no such effects. A bilateral abdominal vagotomy performed before the infusions totally abolished both the insulin and blood flow response to glucose infusion. The absence of an increased islet blood flow in response to glucose infusion in the denervated, transplanted pancreas was a further indication of the crucial importance of the regulation of islet blood flow by the vagus nerves. It is concluded that intestinal glucoreceptors participate in the mediation of glucose-induced islet blood flow increase.

pancreatic islets; intestinal glucoreceptors; pancreatic islet blood flow; denervation; vagus nerve

The proportion of blood perfusing the pancreatic islets is very high and constitutes 7–10% of the whole pancreatic blood flow, despite the islets contributing only ~1% to the pancreatic volume (4, 8). The blood flow to the endocrine pancreas is regulated independently from that of the exocrine part to optimize the oxygen and nutrient supply to the needs of the respective tissues (see Ref. 8). This intrapancreatic regulation of blood flow is mediated by complex interactions between locally produced vasodilators and vasoconstrictors, gastrointestinal hormones, and the nervous system (8, 11, 23). Pancreatic islet blood flow is usually, but not necessarily, increased whenever insulin release is stimulated (8, 10). The single most important factor mediating this response seems to be stimulation of glucoreceptors in the central nervous system that relay signals to the islets through a vagal cholinergic mechanism (11, 13). It should be noted, however, that glucoreceptors are present not only in the brain but also in several peripheral organs, such as the intestines (2, 15-17) and the liver (18). Previous studies on hepatic glucoreceptors, which are likely to consist of nerves, have shown that they may affect insulin release from the pancreatic islets through a vagal mechanism (18) and islet blood flow through sympathetic nerves (12).

Stimulation of intestinal glucoreceptors may also lead to an acute increase in insulin release (cf. Refs. 16 and 17) or stimulate the release of gastrointestinal neuropeptides. These peptides may act as hormones and directly potentiate glucose-stimulated insulin secretion, i.e., function as incretins (1, 6, 7). Such incretins, e.g., gastric inhibitory polypeptide, are known to be able to potentiate islet blood flow during hyperglycemia (22). In the present study, we test the hypothesis that intestinal glucoreceptors have a role in the regulation of islet blood flow.

Materials and Methods

Animals. In most of the experiments, male Sprague-Dawley rats obtained from a local breeding colony (Biomedical Center, Uppsala, Sweden) and weighing ~350 g were used. For the pancreas transplants, male inbred Wistar-Furth rats (B & K Universal, Sol lentuna, Sweden) weighing ~300 g were used. The animals had free access to tap water and pelleted food throughout the experimental period. All experiments were approved by the local animal ethics committee at Uppsala University, Uppsala, Sweden.

Surgical preparation. The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg body wt; Apoteksbolaget, Umeå, Sweden), heparinized, and placed on an operating table heated to body temperature (38°C). Polyethylene catheters were inserted into the ascending aorta via the right common carotid artery and also into the right femoral artery and left femoral vein. The aortic catheter was connected to a pressure transducer (PDCR 75/1; Druck, Groby, UK) to allow constant monitoring of the mean arterial blood pressure on a recorder. The abdominal cavity of the animals was then opened with a midline incision. The duodenum and pylorus were exposed. A catheter with a 21-gauge needle at its tip was inserted into the duodenum immediately distal to the pyloric sphincter and connected to an infusion pump (model 355; Sage Instruments, Cambridge, MA). Some of the animals were pretreated with a bilateral abdominal vagotomy or corresponding sham operations ~20 min before commencing the infusion (see below). The vagotomy was performed with the aid of a stereomicroscope to facilitate identification of all nerve fibers.

An intraduodenal infusion (0.1 ml/min for 3 min) with either d-glucose (1 mg/kg body wt) or 3-O-methylglucose (1 mg/kg body wt; Sigma, St. Louis, MO) dissolved in saline or saline alone was then administered. To evaluate the infusion technique, we infused separate animals with saline with 0.4% (wt/vol) trypan blue (Sigma) added. The dye could be seen ~5 cm distal to the infusion site immediately after ending the infusion. After 20 min, all of the small intestine, with the exception of the distal 2–3 cm of the ileum, contained dye. No differences between control or vagotomized rats were seen. Furthermore, in separate animals, blood samples were taken from the portal vein before commencing the infusion and then every second minute for 20 min.

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Pancreaticoduodenal transplantation. This procedure has been described in detail elsewhere (13). Briefly, the donor rat was anesthetized with an intraperitoneal injection of a mixture of chloral hydrate (175 mg/kg body wt) and pentobarbital sodium (40 mg/kg body wt) and placed on a heated operating table. The whole pancreas, together with ~3–4 cm (1 g) of the small intestine, was dissected free from surrounding tissues. Via a catheter in the aorta, the preparation was flushed with 5–7 ml of cold (4°C) UW solution (Belzer UW-CSS; Du Pont Pharmaceuticals, Wilmington, DE) at a pressure of ~100 cm H2O. The warm ischemic time was less than 2 min. The graft was then removed from the animal together with ~1 cm of the aorta, which contained the two arterial vessels of the gland, and stored at 4°C for 60–90 min (cold ischemia time) before being implanted into the recipient.

The recipient animals were anesthetized as described above and placed on a heated operating table. The left kidney was removed, and the pancreaticoduodenal graft was anastomosed to the renal vessels by a nonsuturing cuff technique, as previously described in detail (13, 19). The graft small intestine was sutured end to side to a loop of the colon of the recipient by ~10 sutures with 7–0 silk. After closure of the abdominal wall, the animals were injected subcutaneously with 10 mg doxycycline (Iodocyclin; Leo, Malmö, Sweden) and were observed until they were fully recovered from anesthesia. Blood flow measurements of the native and transplanted pancreas were performed 14 days posttransplantation after the rats with Student’s unpaired t-test.

RESULTS

There were no differences in organ blood flow values between the groups immediately after the end of the 3-min intraduodenal infusion period (Table 1). However, a slight but significant increase in systemic blood glucose concentrations was seen in animals infused with glucose, whereas mean arterial blood pressure and serum insulin concentrations were similar in all groups (Table 1). In separate experiments, no increase in portal glucose concentrations after intraduodenal glucose infusion were seen (data not shown).

When corresponding measurements were performed 20 min after commencing the intraduodenal infusion, an increase in whole pancreatic, islet, and duodenal blood flow was seen after glucose infusion, whereas colonic blood flow was unchanged (Table 2). The osmotic control substance 3-O-methylglucose did not affect any of these organ blood flows (Table 2). No differences in mean arterial blood pressure or blood glucose concentrations were seen when the experimental groups were compared at this time point, whereas the serum insulin concentration was increased in the glucose-infused rats (Table 2).

When a bilateral vagotomy was performed before the intraduodenal infusions, there were no differences in mean arterial blood pressure, blood glucose, or serum insulin concentrations 20 min after the start of the glucose or 3-O-methylglucose infusions (Table 3). Neither could any effects on duodenal, colonic whole pancreatic, or islet blood flow be seen (Table 3).

The whole pancreas-transplanted rats had ~5% decreased body weights 14 days after implantation (Table 4). Mean arterial blood pressure, blood glucose, and serum insulin concentrations were similar in saline- and glucose-infused rats (Table 4). No differences between the blood flow of the native and transplanted pancreas were observed until they were fully recovered from anesthesia.

Table 1. Blood glucose and serum insulin concentrations, mean arterial blood pressure, and organ blood flow values immediately after a 3-min intraduodenal infusion

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Glucose</th>
<th>3-O-MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>4.2 ± 0.2</td>
<td>5.1 ± 0.2*</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>Serum insulin, µU/ml</td>
<td>49 ± 12</td>
<td>56 ± 12</td>
<td>61 ± 13</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>88 ± 3</td>
<td>82 ± 4</td>
<td>91 ± 5</td>
</tr>
<tr>
<td>Pancreatic blood flow, ml·min⁻¹·g⁻¹</td>
<td>0.27 ± 0.03</td>
<td>0.21 ± 0.04</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>Islet blood flow, µl·min⁻¹·g⁻¹</td>
<td>27 ± 3</td>
<td>21 ± 4</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>Duodenal blood flow, ml·min⁻¹·g⁻¹</td>
<td>1.33 ± 0.24</td>
<td>1.14 ± 0.16</td>
<td>1.68 ± 0.20</td>
</tr>
<tr>
<td>Colonic blood flow, ml·min⁻¹·g⁻¹</td>
<td>0.24 ± 0.07</td>
<td>0.29 ± 0.05</td>
<td>0.32 ± 0.06</td>
</tr>
</tbody>
</table>

All values are means ± SE. Intraduodenal infusions (0.1 ml/min) were of saline, 3-glucose (1 mg/kg body wt), or 3-O-methylglucose (3-O-MG; 1 mg/kg body wt). *P < 0.05 compared with saline-infused rats with Student’s unpaired t-test.
organs were seen in saline-infused rats (Table 4). However, after glucose infusion into the native duodenum, whole pancreatic and islet blood flow was increased in the native pancreas but not in the transplant (Table 4).

### DISCUSSION

The findings in the present study suggest yet another mechanism by which glucose can increase islet blood flow in association with an increased insulin release. This confirms the notion that the islets possess a blood flow regulation that is independent of that of the whole pancreas and is adapted to the need for rapid disposal of the released hormones (8). Insulin is the predominant hormone regulating carbohydrate metabolism in mammals, and the major secretagogue for this hormone, namely glucose, has previously been demonstrated to have marked and preferential stimulatory effects on islet blood flow (10). It is known that the islet blood perfusion can be increased by stimulation of glucoreceptors in the central nervous system and that this effect is mediated by a vagal cholinergic mechanism (11). This response is not seen in the denervated, transplanted whole pancreas (13). Furthermore, it was recently demonstrated that stimulation of glucoreceptors within the liver could also increase islet blood flow (12). However, this increase was most likely dependent on noncholinergic, nonvagal nerves.

Most probably, there were no direct effects of glucose on neurons in the central nervous system in the present study, because no changes in blood glucose concentrations were observed. Nor could any increase in portal glucose concentrations be seen, which makes effects on glucoreceptors in the liver less likely as an explanation for the increased islet blood flow seen after glucose administration. Previous morphological studies have demonstrated and traced vagal receptors in the duodenal mucosa (2, 16). Furthermore, several studies have shown the presence of efferent pancreatic vagal fibers with a potential for stimulation of insulin release (1, 3, 14, 21). The importance of the vagus system for glucose-stimulated insulin release (cf. Ref. 15) is confirmed in the present study. One interpretation of our findings is that glucose, when present in the duodenal lumen, stimulates glucoreceptors that send afferent signals via the vagus nerves. These signals are then transmitted through efferent nerves to the pancreas, where they stimulate insulin release and presumably also islet blood flow.

The detailed mechanism for the vagal stimulation of islet blood flow is unknown. It can be envisaged that cholinergic nerves dilate islet afferent arterioles directly (cf. Ref. 8) or that they release a soluble factor(s), e.g., incretins or prostaglandins, which may then cause the increased islet blood perfusion. To investigate the

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### Table 3. Blood glucose concentrations, serum insulin concentrations, mean arterial blood pressure, and organ blood flow values 20 min after a 3-min intraduodenal infusion

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Glucose</th>
<th>3-O-MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Blood glucose concentration, mmol/l</td>
<td>5.1 ± 0.4</td>
<td>5.3 ± 0.4</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>Serum insulin concentration, µU/ml</td>
<td>57 ± 9</td>
<td>65 ± 11</td>
<td>61 ± 12</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>89 ± 7</td>
<td>85 ± 6</td>
<td>90 ± 7</td>
</tr>
<tr>
<td>Pancreatic blood flow, ml·min⁻¹·g⁻¹</td>
<td>0.28 ± 0.05</td>
<td>0.35 ± 0.08</td>
<td>0.29 ± 0.07</td>
</tr>
<tr>
<td>Islet blood flow, µl·min⁻¹·g⁻¹</td>
<td>28 ± 2</td>
<td>30 ± 8</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>Duodenal blood flow, ml·min⁻¹·g⁻¹</td>
<td>1.37 ± 0.33</td>
<td>1.57 ± 0.25</td>
<td>1.26 ± 0.25</td>
</tr>
<tr>
<td>Colonic blood flow, ml·min⁻¹·g⁻¹</td>
<td>0.28 ± 0.07</td>
<td>0.36 ± 0.07</td>
<td>0.26 ± 0.06</td>
</tr>
</tbody>
</table>

All values are means ± SE. Intraduodenal infusions (0.1 ml/min) were of saline, d-glucose (1 mg/kg body wt), or 3-O-ME (1 mg/kg body wt). A bilateral abdominal vagotomy was performed 20 min before start of infusion.
latter possibility, we performed experiments on whole pancreas-transplanted rats, i.e., animals with a denervated pancreas. A vagal release of any circulating factor induced by glucose infusion into the native duodenum would then be likely to also affect the transplanted gland. However, this did not occur. This suggests that an intraduodenal glucose infusion acts via a nervous mechanism that has direct effects on pancreatic blood vessels. Whether the efferent vagal nerves directly influence the tonus of the islet arterial blood vessels is unknown. There is also the possibility that the efferent nerve fibers project on intrapancreatic neurons and that postganglionic nerves then cause the observed circulatory changes.

The results in the present study need not necessarily reflect the presence only of duodenal glucoreceptors but may also be taken to demonstrate the presence of more distally located glucose-sensitive receptors. This is suggested by the findings that no effects on islet blood flow or insulin release were seen immediately after the 3-min glucose infusion, when the most proximal 4–5 cm of intestine were affected by glucose, but only after 20 min, when almost the whole intestine contained the infusedate. However, previous studies have demonstrated that glucoreceptors are more common in the proximal intestine (15). The findings can also be due to the fact that a lag period exists between the activation of duodenal receptors and the insulin and blood flow responses. If this is the case, it would argue for a more complex connection between receptor sensing of the glucose signal to the final nervously mediated effects on islet blood flow. This would be important also from a teleological point of view, because the enhanced islet blood flow and insulin release should coincide with the absorption of glucose, in accordance with the release of incretins (6, 7, 20).

Perspectives

The findings in the present study suggest that glucose increases islet blood flow by stimulating intestinal glucoreceptors. This once again emphasizes the presence of multiple regulatory mechanisms for islet blood perfusion and underlines the importance of the hyperglycemia-induced increase in islet blood flow for the functional activity of the islets.

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