Intraportal glucose infusion and pancreatic islet blood flow in anesthetized rats

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Intraportal glucose infusion and pancreatic islet blood flow in anesthetized rats. Am J Physiol Regulatory Integrative Comp Physiol 279: R1224–R1229, 2000.—The aim of the study was to evaluate whether a selective increase in portal vein blood glucose concentration can affect pancreatic islet blood flow. Anesthetized rats were infused (0.1 ml/min for 3 min) directly into the portal vein with saline, glucose, or 3-O-methylglucose. The infused dose of glucose (1 mg·kg body wt ·min⁻¹) was chosen so that the systemic blood glucose concentration was unaffected. Intraportal infusion of n-glucose increased insulin release and islet blood flow; the osmotic control substance 3-O-methylglucose had no such effect. A bilateral vagotomy performed 20 min before the infusions potentiated the islet blood flow response and also induced an increase in whole pancreatic blood flow, whereas the insulin response was abolished. Administration of atropine to vagotomized animals did not change the blood flow responses to intraportal glucose infusions. When the vagotomy was combined with a denervation of the hepatic artery, there was no stimulation of islet blood flow or insulin release after intraportal glucose infusion. We conclude that a selective increase in portal vein blood glucose concentration may participate in the islet blood flow increase in response to hyperglycemia. This effect is probably mediated via parietal nerves and not through the vagus nerve. Furthermore, this blood flow increase can be dissociated from changes in insulin release.

Intravenous glucose administration leads to a preferential increase in islet blood flow, which is initially mediated by a vagal cholinergic mechanism originating from glucoreceptors in the central nervous system (11, 12). However, glucoreceptors are present also in several peripheral organs, such as the intestines (18) and the intrahepatic blood vessels (22). Previous studies on hepatic glucoreceptors, which presumably consist of nerves (3, 7), have shown that they may affect insulin release from the pancreatic islets (22). Stimulation of these glucoreceptors decreases activity in afferent vagal nerve fibers that project on vagal nuclei in the brain and brain stem (22). Efferent signals are then transmitted through the vagus nerves and relayed to the pancreatic islets. Furthermore, nerves in the adventitia of the hepatic artery, i.e., mainly sympathetic nerves, may transmit signals from the liver to the islets and, thereby, affect insulin release (15, 16). Whether the activity of the latter nerves is increased by glucose is, however, unknown.

In the present study, we investigated whether intraportal glucose infusions, which did not affect systemic blood glucose concentrations, changed pancreatic islet blood flow in concert with insulin release. Glucose was infused intraportally to increase ambient glucose within the liver, without affecting systemic blood glucose concentrations. These experiments were combined with selective denervation procedures to elucidate the mechanisms responsible for the observed glucose-induced increase in islet blood flow.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats, weighing ~325 g, were obtained from a local breeding colony (Biomedical Centre, Uppsala, Sweden) and used in all experiments. The animals had free access to tap water and pelleted food at all times. The experiments were approved by the local Animal Ethics Committee at Uppsala University.

Surgical preparation and pretreatment. The rats were anesthetized with pentobarbital sodium (60 mg/kg body wt ip; Apoteksbolaget, Umeå, Sweden), heparinized, and placed on an operating table heated to body temperature. Polyethylene catheters were inserted into the ascending aorta, via the right common carotid artery, into the right femoral artery and into the left femoral vein. The aortic catheter was con-
nected to a pressure transducer (model 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 opened with a midline incision. Approximately 1 cm of the portal vein, close to the liver hilus, was visualized after blunt dissection. A catheter, with a 21-gauge needle at its tip, was inserted into the portal vein, with care taken to only minimally disturb the flow in the vein, and connected to an infusion pump (model 355, Sage Instruments, Cambridge, MA). The tip of the catheter was located ~5 mm from the liver hilus. Some of the animals were treated with a bilateral abdominal vagotomy immediately below the diaphragm, a surgical denervation of the hepatic artery (15), or corresponding sham operations ~30 min before the blood flow measurements. When the vagotomy was performed, special care was taken to identify all branches from the vagus nerves and to ascertain that no connections with the vagus nerves remained (14). These denervations were performed with the aid of a microscope to facilitate visualization of all nerve fibers. Furthermore, methylatropine (150 μg/kg body wt; ACO, Gothenburg, Sweden) dissolved in 0.2 ml of saline or saline alone was injected into the femoral vein of separate bilaterally vagotomized or sham-operated animals.

An intraportal infusion (0.1 ml/min for 3 min) with D-glucose (1 mg·kg body wt·min⁻¹) or 3-O-methylglucose (1 mg·kg body wt·min⁻¹; Sigma Chemical) dissolved in saline or saline alone was then administered. This infusion was initiated 20 min after the denervation procedures and 7 min after injection of methylatropine or saline.

Experimental groups. The experimental groups were as follows: untreated rats that received intraportal infusions of D-glucose, 3-O-methylglucose, or saline (group A); animals that were pretreated with a bilateral abdominal vagotomy and then infused with D-glucose, 3-O-methylglucose, or saline (group B); animals that were bilaterally vagotomized and given atropine and then infused with D-glucose 3-O-methylglucose, or saline (group C); and rats that were bilaterally vagotomized, with the nerves in the adventitia of the hepatic artery divided, and then infused with D-glucose, 3-O-methylglucose, or saline (group D).

Blood flow measurements. After the 3-min infusion of D-glucose, 3-O-methylglucose, or saline into the portal vein, whole pancreatic blood flow and islet blood flow were measured with a nonradioactive microsphere technique, as previously described in detail (9, 10). Briefly, ~1.5 × 10⁶ nonradioactive microspheres (11 μm diameter; NEN-Trac, Du Pont Pharmaceuticals, Wilmington, DE) suspended in 0.2 ml of saline were injected via the catheter in the ascending aorta. Starting 5 s before the microsphere injections and continuing for 60 s, an arterial reference sample was obtained from the catheter in the right femoral artery at a rate of ~0.25 ml/min. The exact withdrawal rate was determined in each case by weighing the sample. Additional arterial blood samples were obtained and later analyzed for blood glucose concentrations with a blood glucose meter (ExacTech, Baxter Travenol Laboratories, Deerfield, IL) and serum insulin concentrations with RIA (Pharmacia Insulin RIA kit, Pharmacia-Upjohn Diagnostics Sverige, Uppsala, Sweden) with rat insulin (Novo Nordic, Bagsvaerd, Denmark) as a standard.

The rats were killed, and the pancreas, duodenum, colon, and both adrenal glands were removed, blotted, and weighed. The microsphere contents in these organs were determined separately (9). The pancreatic islets were visualized with a freeze-thawing technique, and the number of microspheres in the islets and exocrine parenchyma was counted as previously described in detail (9). The adrenal glands were used as a control to ascertain a complete mixture of the microspheres in the arterial circulation. Only animals with <10% differences in total microsphere content between the two glands were included in the study. The microsphere content of each of the arterial reference samples was determined by transferring the samples to glass microfiber filters and counting the microspheres in a stereomicroscope. The organ blood flow values were calculated as follows: 

\[ Q_{\text{org}} = \frac{Q_{\text{ref}} \times N_{\text{org}}}{N_{\text{ref}}} \]

where \( Q_{\text{org}} \) denotes organ blood flow (ml/min), \( Q_{\text{ref}} \) is withdrawal rate of the reference sample (ml/min), and \( N_{\text{org}} \) and \( N_{\text{ref}} \) represent number of microspheres in the organ and the reference sample, respectively.

Statistical analysis. Values are means ± SE. Probabilities of chance differences between the experimental groups were calculated with one-way ANOVA with Bonferroni’s correction (SigmaStat for Windows, SPSS, Dusseldorf, Germany). \( P < 0.05 \) was considered to be statistically significant.

RESULTS

Mean arterial blood pressure varied between 85 and 100 mmHg in all animals, and no significant differences between any of the groups were seen. Likewise, the blood glucose concentrations (Fig. 1) were not affected by infusion of glucose or 3-O-methylglucose in any of the experimental groups. Vagotomy or vagotomy in combination with atropine administration (groups B and C) did not affect blood glucose concentrations, whereas the values were higher in all animals in group D, i.e., treated with hepatic arterial denervation.

Serum insulin concentrations (Fig. 2) were increased after glucose, but not 3-O-methylglucose, infusion into the control animals (group A). Bilateral abdominal vagotomy, alone or in combination with atropine, as well as arterial denervation (groups B, C, and D) prevented this increase in serum insulin concentrations.

![Fig. 1. Blood glucose concentrations after a 3-min intraportal infusion (0.1 ml/min) of saline (closed bars) or 1 mg·kg body wt·min⁻¹ of either D-glucose (open bars) or 3-O-methylglucose (hatched bars). The animals were otherwise untreated (control), treated with a bilateral abdominal vagotomy (vagotomy), vagotomized and also treated with atropine (vagotomy + atropine), or subjected to hepatic artery denervation (arterial denerv.). Values are means ± SE for 6–8 animals. §P ≤ 0.05 vs. corresponding control animals.](image)
Total pancreatic blood flow (Fig. 3) was unaffected by administration of 3-O-methylglucose in all treatment groups. Glucose infusion caused an increased pancreatic blood flow in groups B and C, whereas no change was seen in group D. Islet blood flow (Fig. 4) was not changed by infusion of 3-O-methylglucose in any of the treatment groups. However, glucose infusion led to a pronounced increase in islet blood flow in groups A, B, and C. Arterial denervation (group D) completely abolished this islet blood flow response to glucose.

Duodenal blood flow (Fig. 5) was unaffected by glucose or 3-O-methylglucose administration in the control animals (group A). In groups B and D, a decrease in duodenal blood flow was seen after saline infusion, whereas in group B a similar decrease was seen after glucose infusion compared with similar animals in group A. The decreases in groups B and C were significant also compared with the other rats in these groups (P ≤ 0.05 for all comparisons). However, in group D, no differences between animals given saline, glucose, or 3-O-methylglucose were seen. Colonic blood flow (Fig. 6) was similar in all animals.
mals were treated as described in Fig. 1 legend. Values are means ± SE for 6–8 animals.

This means that the administra-
tion of glucose concentrations was seen after the intraportal glucose infusions. Furthermore, when similar doses of

glucose were given systemically into a femoral vein in earlier studies, no changes in pancreatic islet blood

flow were observed (11). This means that the adminis-
tration of glucose is likely to have exerted its actions intra-
hepatically or intraportally. Several previous studies have demonstrated an association between stimulation of hepatic glucoreceptors and a potentiation of insulin release (1, 20–22), a notion that was confirmed in the present experiments. Likewise, recent publications have emphasized the importance of glucoreceptors for the control of glucose homeostasis and food intake (17, 19, 20, 30). The anatomic setting for the hepatic glucose sensors is presumably nerve fibers in the portal blood vessels (7) that have the ability to register an increased portal glucose concentration and transmit afferent signals to the brain by decreasing vagal activity (22). Signals from vagal nuclei to the pancreas, which stimulate insulin release, are then transmitted through efferent fibers, also in the vagus nerves, to the pan-
creas (2, 4, 22, 31). Our present findings of a complete prevention of the glucose-induced insulin secretion after vagotomy and atropine injections are in line with these previous results. However, whether the vagot-
omy effects are interrupting afferent or efferent signaling in the nerve is unknown.

Intraportal infusion of glucose into otherwise untreated rats led to an increase in islet blood perfusion associated with an increased release of insulin. This can be interpreted to reflect an association between stimulation of hepatic glucoreceptors by the glucose infusion and a vagally mediated stimulation of insulin release and islet blood flow. However, in contrast to the total abolition of the hepatic glucoreceptor-stimulated increase of insulin release induced by bilateral vagot-
omy, with or without simultaneous atropine administration, whole pancreatic blood flow and islet blood flow were instead further increased after these surgical interventions. This is in marked contrast to the glu-
cose-induced stimulation of islet blood flow mediated by glucoreceptors in the central nervous system (11) or in the intestines (5), which is totally dependent on intact vagus nerves. Under such circumstances, only the islet blood perfusion is affected, whereas whole pancreatic blood flow is not. The present findings therefore suggest that signals that increase islet blood flow after intraportal glucose infusion are more likely to be transmitted through nerve fibers associated with the adventitia of the hepatic artery. In support of this notion, denervation of the hepatic artery totally pre-
vented the increase in islet blood flow seen after stimulation of hepatic glucoreceptors by glucose infusion. The findings of Lindfeldt and co-workers (15, 16), that arterial denervation of the liver affected glucose homeostasis after exercise or partial hepatectomy, sup-
port the existence of such a mechanism. However, the exact anatomic identity and neurotransmitter content of the nerves involved in such a response are unknown.

The possibility that glucose-induced stimulation of hepatic nerves leads to a release of other gastrointes-
tinal hormones, which then cause the observed effects on islet blood flow, cannot be excluded. It is known that the nervous system may have profound effects on gastro-
tintestinal endocrine cells (24) and that stimulation of hepatic glucoreceptors may affect intestinal motility.

DISCUSSION

We previously demonstrated that systemic hypergly-
cemia causes a dose- and time-dependent preferential increase in islet blood flow in rats that, at least ini-
tially, depends on selective stimulation of glucorece-
ptors in the central nervous system and is mediated via a vagal, cholinergic mechanism (10, 11). In the present study, we have demonstrated that glucose infusion into the portal vein increases islet blood flow in anesthe-
tized rats, an effect that may be mediated by activation of hepatic glucoreceptors and nerves associated with the hepatic artery. However, changes in hepatic me-
tabolism secondary to the local glucose infusion may also be involved. This finding underlines the crucial importance of glucose for the regulation of islet blood flow and demonstrates a new mechanism by which this can be achieved.

In addition to the changes in total pancreatic and islet blood flow noted in the present study, we also observed changes in duodenal blood flow in some of the groups. Because the intraportal infusion necessitated dissection of the vein, handling of the duodenum was needed in all animals. The extent of these manipula-
tions varied between the animals, and it cannot be excluded that the differences we see in duodenal blood flow may reflect this. The lack of association between the infused substances and the flow values supports this notion.

The dose of glucose (1 mg·kg body wt−1·min−1) was calculated to give a 20–25% increase in portal glucose concentrations. However, no changes in systemic glu-
cose concentrations were seen after the intraportal glucose infusions. Furthermore, when similar doses of glucose were given systemically into a femoral vein in earlier studies, no changes in pancreatic islet blood flow were observed (11). This means that the adminis-
(25) and gastrin secretion (26). However, because previous studies have suggested that gastrointestinal hormones mainly affect whole pancreatic blood flow and usually only induce minor changes in islet blood perfusion (8), we consider this unlikely.

We cannot exclude possible nonspecific effects of the vagotomy procedure, affecting afferent or efferent fibers, which may influence islet blood flow in the present study. However, it is unlikely that such effects can explain the glucose-induced changes in islet blood flow.

The intrahepatic portal vascular bed also contains, in addition to glucoreceptors, nerves sensitive to, e.g., sodium, osmolality, pressure, and amino acids (6, 13, 19, 23, 28, 29), which may affect the present measurements. However, the lack of any effects of 3-O-methylglucose seems to rule out any possible effects of osmotic receptors. The infusate contained no amino acids, which makes it unlikely that stimulation of such receptors was of importance in the present study. All solutions contained saline, but in physiological concentrations, which makes stimulation of such receptors unlikely to cause any of the observed differences between the experimental groups.

**Perspectives**

This newly described mechanism causing an increased blood flow to the pancreas and islets after intraportal infusion of glucose once again underlines the importance of the islet blood flow increase seen in association with systemic or regional hyperglycemia within the digestive tract or central nervous system. It can be envisaged that the blood flow increase facilitates the increased islet metabolism and the disposal of the secreted islet hormones. The nervous mechanisms responsible for the islet blood flow increase seen in the present study are not vagal, in contrast to all previously demonstrated glucose-induced islet blood flow stimulations. This once again stresses the versatility of the body’s mechanisms for maintaining a high islet blood flow when the functional demands of an increased hormonal release are present.

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