Pancreatic islet blood flow in conscious rats during hyperglycemia and hypoglycemia

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Address for reprint requests and other correspondence: M. Iwase, Dept. of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu Univ., Maidashi 3–1-1, Higashi-ku, Fukuoka 812–8582, Japan (E-mail: iwase@intmed2.med.kyushu-u.ac.jp).

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Iwase, M., K. Tashiro, Y. Uchizono, D. Goto, and M. Yoshinari. Pancreatic islet blood flow in conscious rats during hyperglycemia and hypoglycemia. Am J Physiol Regulatory Integrative Comp Physiol 280: R1601–R1605, 2001.—Anesthesia affects general hemodynamics and regulation of organ perfusion. We used colored microspheres to measure pancreatic islet blood flow in conscious rats at two time points, during either hyperglycemia or hypoglycemia. This method, using black and green microspheres, was validated by comparison with previous microsphere experiments and by lack of effect of a nonmetabolizable glucose analog, 3-O-methylglucose, on islet perfusion. Basal and glucose-stimulated islet blood flow levels were similar in pentobarbital sodium-anesthetized and conscious rats. However, the basal distribution of pancreatic blood flow was altered by anesthesia (fractional islet blood flow 5.8 ± 0.4% in conscious rats, 7.9 ± 0.8% in pentobarbital-anesthetized rats, P < 0.05). Insulin-induced hypoglycemia significantly increased whole pancreatic blood flow in conscious rats, whereas islet blood flow remained unchanged and fractional islet blood flow was decreased (5.8 ± 0.5% in the basal state, 4.2 ± 0.4% during hypoglycemia, P < 0.001). Methylatropine pretreatment significantly increased islet blood flow during hypoglycemia by 181%. This result suggests that prevention of hypoglycemia-induced increase in islet perfusion may be mediated, at least in part, by a cholinergic, vagal muscarinic mechanism.

Methods

Animals and surgery. Eight-week-old male Sprague-Dawley rats of ∼300 g body weight (n = 55, Kyudo, Fukuoka, Japan) were used for the experiments. For anesthesia, pentobarbital sodium (Abbott Laboratories, Chicago, IL) was injected intraperitoneally at a dose of 50 mg/kg. Microsphere experiments were performed in the anesthetized rats after stabilization of blood pressure and body temperature. Body temperature was maintained at 37.5°C using a body temperature controller (Fine Science Tools, Foster City, CA). Polyethylene tubing (PE-50) was implanted in the ascending aorta via the right carotid artery, and the femoral artery was cannulated. Blood pressure was monitored by a pressure transducer (Nihon Koden, Tokyo, Japan) connected to the arterial catheter. Heparin (100 IU; Shimizu, Shizuoka, Japan) was administered via the right jugular vein. When conscious rats were used, the intravenous and arterial catheters were exteriorized through the back of the neck, filled with heparinized saline, and each was plugged with a pin. Experiments were performed at least 72 h postoperatively. The microsphere experiments were performed in an unrestrained state after administration of heparin via the carotid catheter and stabilization of arterial blood pressure. All experiments were performed according to the guidelines of the Animal Experimentation Ethics Committee of Kyushu University.

NEN vs. E-Z colored microspheres. Pentobarbital-anesthetized rats (n = 10) were used for comparison of two types of
colored microspheres (NEN-TRAC microsphere, Du Pont, Wilmington, DE; E-Z TRAC microsphere, Interactive Medical Technology, San Diego, CA). NEN microspheres have been used previously for measurement of islet blood flow in rats (10). According to the information supplied by the manufacturers, the size distribution of NEN microspheres is 11.4 ± 0.1 (± SD) μm and that of E-Z microspheres is 10.0 ± 0.23 μm, and the density of NEN microspheres is 1.4 g/ml, whereas that of E-Z microspheres is 1.05 g/ml. The latter is closer to the density of red blood cells (1.10 g/ml). NEN microspheres are black only, whereas E-Z microspheres are available in green, orange, red, blue, yellow, and black. Orange, red, and yellow E-Z microspheres were invisible in the blood reference sample, and black and blue microspheres were indistinguishable in pancreatic tissue. Therefore, we used green and black microspheres in our study. Black NEN microspheres and green E-Z microspheres were used to compare NEN and E-Z microspheres. The order of administration was switched for every experiment. Islet blood flow was measured according to the method of Jansson and Hellerström (12). Microspheres were suspended in saline and sonicated before injection. Microspheres (400,000) were then injected and flushed with 350 μl saline into the ascending aorta over 25 s. The reference blood sample was withdrawn from the femoral artery catheter into a syringe at a rate of 0.5 ml/min using a constant-withdrawal pump (model 120, KD Scientific, Boston, MA) commencing 10 s before injection of the microspheres.

Blood samples were obtained for determination of blood glucose and serum immunoreactive insulin (IRI). Blood glucose was measured by the electrode method (Glutest Ace, Kyotodaichikagaku, Kyoto, Japan), and serum IRI was measured with an ELISA commercial kit using a rat insulin standard (Morinaga, Yokohama, Japan). The rats were then killed, and the whole pancreas, entire duodenum, and adrenal glands were carefully removed, blotted, and weighed. The whole pancreas was carefully dissected free of fat and lymph nodes under a stereomicroscope (Leica MZ8, Leica, Heerbrugg, Switzerland). Each pancreas was cut into small pieces and placed between object slides. Pancreatic specimens were treated with a freeze-thawing technique to facilitate visualization of microspheres and pancreatic islets. The volume of the islets as a percentage of the pancreatic tissue was determined using the point-counting method (4). Intersections of overlapping islets were counted at a magnification of ×40, and a total of 36 ± 1 different fields was counted in each pancreas (corresponding to 4,329 ± 79 points; n = 55). Whole pancreas and duodenum were digested overnight with 2 mol/l NaOH at 70°C. The microsphere content of each organ and reference blood sample was determined by transferring parts of the samples, after vigorous stirring, to glass microfiber filters (GPA, Whatman, Kent, UK) and counting the microspheres under a stereomicroscope. By determining the number of microspheres present in the organ and arterial reference samples, blood flow values were calculated according to the formula: \[ Q_{\text{org}} = N_{\text{obs}} \times 0.5/N_{\text{ref}}, \] where \( Q_{\text{org}} \) is the organ blood flow (ml/min), 0.5 is the withdrawal rate of the reference sample (ml/min), \( N_{\text{obs}} \) is the number of microspheres in the organ, and \( N_{\text{ref}} \) is the number of microspheres in the reference sample. A difference of <10% in microsphere content of the two adrenal glands was taken to indicate sufficient arterial mixing of microspheres. When the islet blood flow was expressed per islet weight, islet weight was estimated by multiplying the pancreatic weight by the islet volume fraction of the whole pancreas. Fractional islet blood flow was expressed as a percentage of whole pancreatic blood flow.

**Evaluation of the two-color microsphere method.** Black and green E-Z microspheres were used in anesthetized rats (n = 8). The order of administration was switched every experiment. A nonmetabolizable glucose derivative, 3-O-methylglucose (5 g/kg; Sigma Chemical, St Louis, MO), was injected via the carotid artery catheter 8 min after the first microsphere injection and was followed 3 min later by the second microsphere injection.

**Comparison of effect of hyperglycemia on islet perfusion in anesthetized and conscious rats.** Islet blood flow in basal and glucose-stimulated states was measured using the two-color microsphere method in pentobarbital-anesthetized (n = 10) and conscious (n = 9) rats. Glucose solution (50%; 5 g/kg) was administered via the carotid artery catheter 30 min before the first microsphere injection to block peripheral parasympathetic, muscarinic receptors. Methylatropine does not readily cross the blood-brain barrier and thus does not impair central nervous system muscarinic cholineric neurotransmission. This dose of methylatropine did not significantly inhibit the increase of plasma glucagon observed during insulin-induced hypoglycemia (7).

**Statistical analysis.** Values are expressed as means ± SE. Student’s paired t-test was used for comparison of data from the same animal. Student’s unpaired t-test was used for comparison of two groups, and ANOVA and Scheffé’s test as a post hoc test were used for comparison of multiple groups. Differences were considered significant if the P value was <0.05.

**RESULTS**

The two-color-microsphere method was evaluated using two approaches: by comparison with NEN microspheres and by using the nonmetabolizable glucose analog 3-O-methylglucose, which is known to have no effect on islet perfusion (Table 1) (12). Blood glucose, serum IRI, and mean arterial blood pressure were similar irrespective of whether NEN microspheres or E-Z microspheres were used for experiments. In addition, there were no significant differences in whole pancreatic blood flow, islet blood flow corrected for pancreatic weight, or estimated islet weight, fractional islet blood flow, or duodenal blood flow. Blood glucose, serum IRI, and mean blood pressure remained unchanged after 3-O-methylglucose injection. Blood flow measurement with black and green E-Z microspheres revealed that intravenous injection of 3-O-methylglucose had no effect on whole pancreatic, islet, fractional, or duodenal blood flows.

As shown in Table 2, both blood glucose and serum IRI increased in anesthetized and conscious rats after glucose injection. Blood glucose, however, was significantly higher, and serum IRI was lower after a glucose load in pentobarbital-anesthetized rats than in conscious rats. Mean blood pressure did not differ signifi-
cantly between anesthetized and conscious rats. Whole pancreatic blood flow significantly increased in both groups after glucose injection, and there were no significant differences between the two groups. Islet blood flow increased in anesthetized rats by 111% and in conscious rats by 150%. No differences were observed in islet blood flow corrected for pancreatic or islet weight between anesthetized and conscious rats. In the basal state, fractional islet blood flow was significantly higher in anesthetized rats than in conscious rats. This parameter rose in both groups after glucose injection, but it did not differ significantly between them. Duodenal blood flow was significantly reduced in anesthetized rats compared with conscious rats in the basal state, whereas it did not differ after glucose injection.

After insulin administration to conscious rats (Table 3), blood glucose was reduced, but blood pressure remained unchanged. Whole pancreatic blood flow and duodenal blood flow were significantly increased during hypoglycemia. However, islet blood flow corrected for pancreatic or islet weight was not affected by insulin-induced hypoglycemia, whereas fractional islet blood flow was significantly decreased. Methylatropine pretreatment did not significantly alter blood glucose or blood pressure. However, basal pancreatic and islet blood flows were significantly reduced by pretreatment with methylatropine. During hypoglycemia, however, whole pancreatic and islet blood flows were significantly increased in methylatropine-treated rats by 239% and 181%, respectively. Islet blood flow was significantly elevated compared with that without methylatropine pretreatment. No significant changes were observed in fractional islet or duodenal blood flow during hypoglycemia after methylatropine pretreatment.

**DISCUSSION**

E-Z microspheres are commercially available in six different colors at present; however, only the combination of black and green microspheres was applicable to measurement of islet perfusion in our study. E-Z microspheres differ from NEN microspheres in that they have a larger size variation and a density closer to that of red blood cells. However, we demonstrated very similar islet blood flow with either NEN or E-Z microspheres. Jansson (10) reported that basal islet blood flow in rats was 40–70 μl·min⁻¹·g pancreas⁻¹ using NEN microspheres, a value about one-half that which we obtained in the experiments reported here. However, when islet blood flow was corrected for islet tissue weight, our result is similar to that reported by Jansson’s group, 6.1 ml·min⁻¹·g islet tissue weight⁻¹ in anesthetized normal Wistar rats (5). This indicates that islet blood flow is more appropriately expressed as corrected for islet tissue weight. The comparison of NEN and E-Z microspheres and the lack of effects of 3-O-methylglucose on islet blood flow validated the two-color microsphere method for measurement of islet perfusion in rats.

Pentobarbital sodium has suppressant actions on the cardiovascular system with reduction in cardiac output, depression of sympathetic and parasympathetic functions, and lowering of blood pressure in rats (19).

### Table 1. Evaluation of 2-color microsphere method in anesthetized rats

<table>
<thead>
<tr>
<th>Microspheres (n = 10)</th>
<th>3-O-Methylglucose (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NEN</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>5.7 ± 0.1</td>
</tr>
<tr>
<td>Serum IRI, ng/ml</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>106 ± 4</td>
</tr>
<tr>
<td>Whole pancreatic blood flow, ml·min⁻¹·g⁻¹</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Islet blood flow, μl·min⁻¹·g islet tissue wt⁻¹</td>
<td>130 ± 10</td>
</tr>
<tr>
<td>Fractional islet blood flow, %</td>
<td>8.5 ± 0.6</td>
</tr>
<tr>
<td>Duodenal blood flow, ml·min⁻¹·g⁻¹</td>
<td>2.7 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n is no. of rats. *Black NEN microspheres and green E-Z microspheres were used in the same rats; †black and green E-Z microspheres were used in the same rats.

### Table 2. Basal and glucose-stimulated islet blood flow in anesthetized and unanesthetized rats

<table>
<thead>
<tr>
<th></th>
<th>Anesthetized Rats (n = 10)</th>
<th>Unanesthetized Rats (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Glucose</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>7.2 ± 0.4</td>
<td>17.8 ± 0.6b,e</td>
</tr>
<tr>
<td>Serum IRI, ng/ml</td>
<td>1.3 ± 0.2</td>
<td>16.2 ± 2.3c,e</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>114 ± 4</td>
<td>113 ± 4</td>
</tr>
<tr>
<td>Whole pancreatic blood flow, ml·min⁻¹·g⁻¹</td>
<td>1.9 ± 0.2</td>
<td>2.7 ± 0.2d,e</td>
</tr>
<tr>
<td>Islet blood flow, μl·min⁻¹·g pancreas⁻¹</td>
<td>142 ± 15</td>
<td>283 ± 30</td>
</tr>
<tr>
<td>Islet blood flow, μl·min⁻¹·g islet tissue wt⁻¹</td>
<td>6.3 ± 0.5</td>
<td>12.8 ± 1.0e</td>
</tr>
<tr>
<td>Fractional islet blood flow, %</td>
<td>7.9 ± 0.8e</td>
<td>10.8 ± 0.9e</td>
</tr>
<tr>
<td>Duodenal blood flow, ml·min⁻¹·g⁻¹</td>
<td>1.9 ± 0.1e</td>
<td>2.5 ± 0.2e</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, bP < 0.01 vs. unanesthetized rats; cP < 0.05, dP < 0.01, eP < 0.001 vs. basal.
Conversely, surgical stresses such as neck surgery and catheter implantation activate the sympathetic nervous system and offset the hypotensive effects of pentobarbital sodium. Hindlycke and Jansson (9) investigated the effects of various anesthetic agents on islet perfusion. They found that islet blood flow was increased by chloral hydrate but markedly decreased by ketamine and xylazine compared with pentobarbital or thiobutabarbital anesthesia. We demonstrated that islet blood flow was not affected by pentobarbital anesthesia compared with conscious rats. This result was somewhat unexpected, because pancreatic islets are richly innervated (1) and islet perfusion is regulated by a neural mechanism (10, 13). Previous studies have shown that nitric oxide synthase inhibitors caused a marked reduction in islet perfusion (16, 21), suggesting that nitric oxide released from endothelial cells may locally regulate islet blood flow. The finding that anesthesia and surgical stress did not affect islet perfusion indicated that local regulation of islet perfusion might be more predominant than neural regulation during hyperglycemia.

Hypoglycemia induces systemic hemodynamic changes including increased cardiac output; increased blood flow to brain, skeletal muscle, and foregut; and decreased blood flow to kidneys and spleen (8). With regard to the splanchnic circulation, blood flow in the superior mesenteric artery increased by 53% in healthy subjects during hypoglycemia, as demonstrated using a Doppler technique (2). This may result in an increased metabolic fuel supply for hepatic gluconeogenesis via the portal system. This is consistent with increased blood flow documented in the whole pancreas and duodenum during hypoglycemia in the present study. Sparrow and Beckingham (20) reported that hypoglycemia increased pancreatic islet blood flow by 221% in pentobarbital-anesthetized rats, whereas whole pancreatic blood flow remained unchanged. They suggested that the increase in islet perfusion might be explained by increased activity of islet α-cells to secrete glucagon during hypoglycemia, although α-cells constitute only a minor proportion of islet cells as a mantle. In contrast to this, our results demonstrated that islet perfusion remained unchanged, whereas whole pancreatic blood flow was increased and fractional islet blood flow was decreased during hypoglycemia. This discrepancy with the previous results may be due to the anesthesia used in the previous study. Because the remaining β-cells are suppressed to decrease endogenous insulin secretion during hypoglycemia, this is compatible with the unchanged islet blood flow and decreased fractional islet blood flow observed in our study.

The vagus nerve participates in the regulation of whole pancreatic and islet perfusion (10). In the present study, whole pancreatic and islet blood flow decreased by peripheral blockade of parasympathetic nerves with methylatropine. Atropine directly suppresses pancreatic exocrine and endocrine secretion in the nonfasting state (1, 18), which may be associated with the reduced whole pancreatic and islet blood flow. In previous studies, however, basal whole pancreatic and islet perfusion were not affected by atropine administration or vagotomy in anesthetized rats (13). This discrepancy may also be explained by the use of anesthetic agents. Because hypoglycemia strongly stimulates the autonomic nervous system, peripheral cholinergic muscarinic blockade manifests as adrenergic activation. In general, vagal cholinergic nerves stimulate islet blood flow, whereas adrenergic effects on islet perfusion are complex (10). Norepinephrine infusion and β2-adrenoceptor stimulation induced a decrease in islet blood flow, and α-receptor stimulation and isoproterenol infusion induced an increase in islet perfusion (11, 15, 17). The finding that pretreatment with methylatropine increased islet perfusion during hypoglycemia is in marked contrast to the finding that atropine or vagotomy abolished a glucose-stimulated increase in islet perfusion (13). However, the effect of vagal muscarinic blockade was specific to islet blood flow in the latter but not in the former. The vagal nervous system is known to mediate the hypoglycemia-induced increase in islet blood flow (13). Therefore, it is interesting that the vagal nervous system also apparently prevents an increase in islet perfusion during hypoglycemia.

In conclusion, we developed a two-color microsphere technique in conscious rats. Basal and glucose-stimulated islet blood flow levels were similar in pentobarbital-anesthetized and conscious rats. However, the basal distribution of blood flow within the pancreas was altered by anesthesia. Insulin-induced hypoglycemia increased whole pancreatic blood flow, whereas it failed to change islet blood flow, and blood flow diversion to pancreatic islets was decreased in conscious rats. Because pretreatment with methylatropine in-
increased islet blood flow during hypoglycemia, prevention of this increase in islet perfusion during hypoglycemia may be mediated, at least in part, by a cholinergic, vagal muscarinic mechanism.

**Perspectives**

Pancreatic islet blood flow may be measured in the conscious state using colored microspheres at two time points. The lack of effect of anesthesia on a glucose-induced increase in islet perfusion suggests that this phenomenon may be essential in response to acute hyperglycemia. Islet blood flow appears to be regulated according to the activity of pancreatic islets during hyperglycemia or hypoglycemia in a manner independent of the exocrine pancreas. This once again emphasizes the importance of regulation of pancreatic islet perfusion in islet functions.

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