# $K_{ATP}$ Channels and Pancreatic Islet Blood Flow in Anesthetized Rats

## Increased Blood Flow Induced by Potassium Channel Openers

**Leif Jansson,[1] Mikael Kullin,[2] F. Anders Karlsson,[2] Birgitta Bodin,[1] John Bondo Hansen,[3] and Stellan Sandler[1]**



**$K_{ATP}$ channels are important for insulin secretion and depolarization of vascular smooth muscle. In view of the importance of drugs affecting $K_{ATP}$ channels in the treatment of diabetes, we investigated the effects of these channels on splanchnic blood perfusion in general and pancreatic islet blood flow in particular. We treated anesthetized Sprague-Dawley rats with the $K_{ATP}$ channel openers diazoxide or NNC 55-0118 or the $K_{ATP}$ channel closer glipizide. Both diazoxide and NNC 55-0118 dose-dependently increased total pancreatic and islet blood flow in the presence of moderate hyperglycemia, but had no effects on the blood perfusion of other splanchnic organs. Diazoxide markedly lowered the mean arterial blood pressure and thus increased vascular conductance in all organs studied. NNC 55-0118 had much smaller effects on the blood pressure. Glipizide did not affect total pancreatic blood flow, but decreased islet blood flow by 50% in the presence of hypoglycemia. We conclude that $K_{ATP}$ channels actively participate in the blood flow regulation of the pancreatic islets and that substances affecting such channels may also influence islet blood flow. *Diabetes* 52:2043–2048, 2003**

The vascular system of the pancreatic islets is both anatomically and functionally autonomous from that of the exocrine parenchyma (1,2). Previous studies have demonstrated that islet blood perfusion is adapted to the high metabolic demands of the endocrine cells and that the islets are able to regulate their flow to the needs for hormone release (1). To achieve this minute regulation, the islet blood perfusion is influenced by both metabolic and nervous signals (1,3). Adenosine seems to be of major importance for metabolic blood flow responses in the islets (3) and, for example, in retina (4) and heart (5). In the latter organs, the effects of adenosine are mediated by $K_{ATP}$ channels.

In addition to being present in vascular smooth muscle (6), $K_{ATP}$ channels are of crucial importance for insulin release by maintaining a hyperpolarization of normal β-cells. Thus, when the potassium channels are closed by increased cellular concentrations of ATP (e.g.,. in association with β-cell glucose metabolism, an altered ion flux leads to depolarization). This opens voltage-gated $Ca^{2+}$ channels, which increase cytosolic $Ca^{2+}$ concentrations, thereby favoring exocytosis (7,8). Both $K_{ATP}$ channel closers and openers are available, and the former, i.e., sulfonylureas, have widespread use as a treatment for type 2 diabetes.

Recently, $K_{ATP}$ channel openers, like diazoxide, have also become of interest in view of the concept of β-cell rest as an option in the initial treatment of type 1 diabetes. An argument for this approach is that inhibition of insulin secretion lowers the expression of β-cell autoantigens (9), which may diminish the immune-mediated assault characteristic of type 1 diabetes. However, most of the early $K_{ATP}$ channel openers were associated with marked hypotension, which made their continuous and frequent clinical use difficult. Recently, more β-cell–specific drugs with only minor effects on blood pressure have been developed (10,11).

Given the combined vasoactive and insulin-releasing properties associated with activation or inhibition of $K_{ATP}$ channels, we deemed it of interest to investigate their effects on pancreatic islet blood perfusion. To achieve this, we used diazoxide, which opens both β-cell and vascular smooth muscle $K_{ATP}$ channels as well as the more β-cell–specific $K_{ATP}$ channel opener NNC 55-0118 (10,11). Furthermore, we also examined the effects of a $K_{ATP}$ channel closer, glipizide. Our findings suggest that opening of $K_{ATP}$ channels in β-cells are associated with a marked increase in total pancreatic and islet blood flow, with a preference for the exocrine parenchyma.

### RESEARCH DESIGN AND METHODS

**Animals.** Male Sprague-Dawley rats, weighing 325–350 g, were obtained from a local breeding colony (Biomedical Center, Uppsala, Sweden) and were used in all experiments. All animals had free access to tap water and pelleted rat food. The use of animals was in accordance with international guidelines (NIH 85-23) and were approved by the local animal ethics committee at Uppsala University.

From the [1]Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden; the [2]Department of Medical Sciences, Uppsala University, Uppsala, Sweden; and [3]Novo Nordisk A/S, Måløv, Denmark.

Address correspondence and reprint requests to Dr. Leif Jansson, Department of Medical Cell Biology, Biomedical Centre, Box 571, SE-751 23 Uppsala, Sweden. E-mail: leif.jansson@medcellbiol.uu.se.

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ELISA, enzyme-linked immunosorbent assay; SUR, sulfonylurea receptor; VSMC, vascular smooth muscle cell.

TABLE 1
Measurements were made before (0 min) or 20 min after an intravenous injection of 2 ml/kg saline or diazoxide (10, 20, or 30 mg/kg) in anesthetized Sprague-Dawley rats

| Treatment | Saline | Diazoxide (10 mg/kg) | Diazoxide (20 mg/kg) | Diazoxide (30 mg/kg) |
|---|---|---|---|---|
| *n* | 7 | 8 | 8 | 8 |
| Blood glucose (mmol/l) | | | | |
| 0 min | 6.6 ± 0.1 | 6.8 ± 0.2 | 6.3 ± 0.1 | 6.4 ± 0.2 |
| 20 min | 6.8 ± 0.2 | 9.1 ± 0.3* | 10.6 ± 0.6* | 9.6 ± 0.5* |
| Serum insulin (ng/ml) | 1.17 ± 0.17 | 0.82 ± 0.09 | 0.74 ± 0.11 | 0.12 ± 0.03* |
| Mean arterial blood pressure (mmHg) | | | | |
| 0 min | 122 ± 4 | 120 ± 4 | 118 ± 4 | 116 ± 4 |
| 20 min | 120 ± 5 | 93 ± 4*† | 69 ± 4*† | 60 ± 1*†‡ |

Data are means ± SE. *$P < 0.001$ vs. saline-injected rats; †$P < 0.001$ vs. animals given diazoxide 10 mg/kg; ‡$P < 0.01$ vs. animals given diazoxide 20 mg/kg. All comparisons were made with ANOVA using Bonferroni's correction.

**Blood flow measurements after diazoxide administration.** The rats were anesthetized with an intraperitoneal injection of thiobutabarbital sodium (130 mg/kg body wt, Inactin; Research Biochemicals International, Natick, MA) and placed on a heated operating table to maintain body temperature. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd., Groby, U.K.), whereas the latter was used to infuse Ringer solution (5 ml · kg body $wt^{-1}$ · $h^{-1}$) to substitute for fluid losses. When the blood pressure had remained stable for at least 15 min an intravenous injection of diazoxide (Hypertstat; Schering-Plough AB, Stockholm, Sweden; 10, 20, or 30 mg/kg body wt) or the corresponding volume (2 ml/kg body wt) of saline was given. The blood flow measurements were then performed 20 min later.

The arterial blood perfusion of the whole pancreas, islets, duodenum, colon, adrenal glands, and kidneys was then measured with a microsphere technique (12). Briefly, a total of 1.5–2.0 × $10^5$ nonradioactive microspheres (EZ-Trac; Triton Microspheres, San Diego, CA), with a diameter of 10 μm, was injected via the catheter with its tip in the ascending aorta during 10 s. Starting 5 s before the microsphere injection, and continuing for a total of 60 s, an arterial blood sample was collected by free flow from the catheter in the femoral artery at a rate of ~0.40 ml/min. The exact withdrawal rate was confirmed in each experiment by weighing the sample. Arterial blood was collected from the carotid catheter for determination of blood glucose and serum insulin concentrations as given below. The animals were then killed, and the pancreas and adrenal glands were removed in toto, blotted, weighed, and treated with a freeze-thawing technique, which visualized the pancreatic islets and microspheres (12). Approximately 100 mg each of the duodenum (around the papilla), colon (descending part), and left kidney (slice through the center) were also removed and treated in the same way. The number of microspheres in these samples were then counted in a microscope equipped with both bright and dark field illumination. The blood flow values were calculated according to the formula $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$, where $Q_{org}$ is organ blood flow (ml/min), $Q_{ref}$ is withdrawal rate of the reference sample, $N_{org}$ is number of microspheres present in the organ, and $N_{ref}$ is number of microspheres in the reference sample. Blood flow values based on the microsphere content of the adrenal glands were used to confirm that the microspheres were adequately mixed in the circulation. A difference <10% in the blood flow values was taken to indicate sufficient mixing.

The number of microspheres in the arterial reference sample was determined by sonicating the blood, then transferring samples to glass microfiber filters (pore size <0.2 μm), and then counting the number of microspheres in the same microscope as referred to above.

**Blood flow measurements after administration of NNC 55-0118.** The surgical preparation was performed as given above. NNC 55-0118 (6-chloro-3-isopropylamino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide; Novo Nordisk A/S, Måløv, Denmark) was administered intravenously (3 mg/kg body wt) 20 min before the blood flow measurements. Control animals were given the same volume (2 ml/kg body wt) of vehicle alone. The blood flow measurements were performed in the same organs and with the microsphere technique referred to above.

**Blood flow measurements after administration of glipizide.** The surgical preparation was performed as given above. Glipizide (Sigma, St. Louis, MO) was dissolved in a 50% solution of DMSO and then administered intravenously (25 μg/kg body wt) 45 min before the blood flow measurements. This time point was chosen since it was the earliest providing a significant decrease in blood glucose concentrations. Control animals were given the same volume (0.5 ml/kg body wt) of vehicle alone. The blood flow measurements were performed with the microsphere technique referred to above. However, only the blood perfusion to the whole pancreas, islets, and the adrenal glands was determined in these animals.

**Measurements of blood glucose and serum insulin concentrations.** Blood glucose concentrations were measured with test reagent strips (Medisense; Svenska MediSense, Solna, Sweden). Serum immunoreactive insulin concentrations were measured with enzyme-linked immunosorbent assay (ELISA) (Rat Insulin ELISA; Mercodia AB, Uppsala, Sweden).

**Statistical calculations.** All values are given as means ± SE. Probabilities (*P*) of chance differences were calculated with Students unpaired *t* test or ANOVA with Bonferroni's correction (SigmaStat; SSSPD, Erfart, Germany) A value of $P < 0.05$ was considered to be statistically significant.

## RESULTS

**Administration of diazoxide.** As expected, diazoxide induced a marked, dose-dependent decrease in mean arterial blood pressure (Table 1). Blood glucose concentrations were increased to the same extent by all doses of diazoxide, whereas serum insulin concentrations were decreased only by the highest dose (30 mg/kg; Table 1). There was also a tendency to a decline in serum insulin when diazoxide was given at a dose of 20 mg/kg, although it did not attain statistical significance ($P = 0.056$).

Total pancreatic blood flow was increased by all doses of diazoxide, but no further increase was seen when the dose was raised from 20 to 30 mg/kg (Fig. 1). Islet blood flow was increased following administration of the two highest doses of diazoxide, with the most pronounced effect seen in animals receiving 20 mg/kg (Fig. 2). When islet blood flow was calculated as a fraction of total

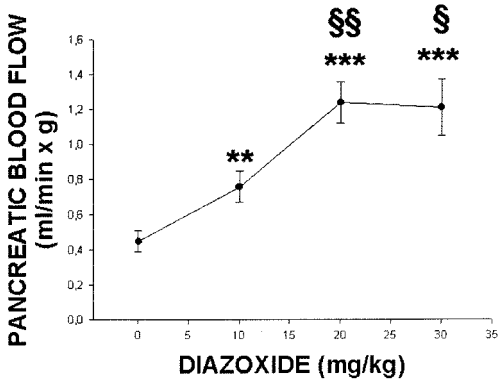


**FIG. 1. Total pancreatic blood flow 20 min after intravenous administration of different doses of diazoxide or saline (concentration 0) to anesthetized Sprague-Dawley rats. Values are means ± SE for 7–8 experiments. **$P < 0.01$, *** $P < 0.001$ when compared with the animals receiving only saline. §$P < 0.05$, §§$P < 0.01$ when compared with animals receiving diazoxide 10 mg/kg. All comparisons were made with ANOVA using Bonferroni's correction.**

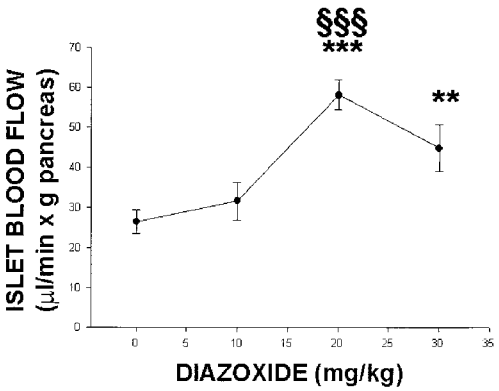


**FIG. 2. Pancreatic islet blood flow 20 min after intravenous administration of different doses of diazoxide or saline (concentration 0) to anesthetized Sprague-Dawley rats. Values are means ± SE for 7–8 experiments. **$P < 0.02$, ***$P < 0.001$ when compared with the animals receiving only saline. §§§$P < 0.001$ when compared with animals receiving diazoxide 10 mg/kg. All comparisons were made with ANOVA using Bonferroni's correction.**

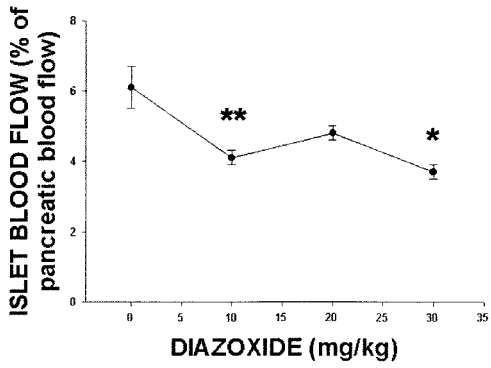


**FIG. 3. Fractional islet blood flow 20 min after intravenous administration of different doses of diazoxide or saline (concentration 0) to anesthetized Sprague-Dawley rats. Values are means ± SE for 7–8 experiments. *$P < 0.05$, **$P < 0.01$ when compared with the animals receiving only saline. All comparisons were made using ANOVA with Bonferroni's correction.**

pancreatic islet blood flow, a decrease was seen when 10 or 30 mg/kg of diazoxide was given (Fig. 3). When 20 mg/kg diazoxide was given, the difference was not significant ($P = 0.055$). This indicates that diazoxide caused a redistribution of the blood perfusion within the pancreas in favor of the exocrine compartment.

The blood perfusion of the kidneys and adrenal glands was unaffected by diazoxide (Table 2). The highest dose of diazoxide induced an increase in both duodenal and colonic blood flow when compared with animals given 10 mg/kg, but not when compared with control rats (Table 2).

Because all doses of diazoxide decreased mean arterial blood pressure, we calculated the vascular conductance of the different organs in which blood flow measurements were made (Table 3). As can be seen, diazoxide at the lowest dose increased pancreatic vascular conductance. The value for adrenal vascular conductance showed an increase, whereas the values for the other organs were unchanged. The two highest doses of diazoxide, however, markedly increased the vascular conductance in all examined organs when compared with both control rats and those given 10 mg/kg diazoxide. No further increase in vascular conductance was seen when rats receiving 30 mg/kg diazoxide were compared with those given 20 mg/kg.

**Administration of NNC 55-0118 (Table 4).** The drug did not affect blood glucose concentrations, but it did decrease serum insulin concentrations and mean arterial blood pressure in the anesthetized rats. No effects on duodenal, colonic, renal, or adrenal blood flow were seen after administration of NNC 55-0118. However, both total pancreatic and islet blood flow were increased after administration of NNC 55-0118. Fractional islet blood flow was not changed after administration of NNC 55-0118 (7.6 ± 0.7 vs. 8.9 ± 0.6%, respectively).

**Administration of glipizide (Table 5).** No effects on mean arterial blood pressure (104 ± 3 vs. 103 ± 1 mmHg in vehicle-treated versus glipizide-treated rats, respectively) were seen. Blood glucose concentrations were decreased 45 min after glipizide administration. Serum insulin concentrations were not measured in these animals due to a technical failure. No effects on total pancreatic blood flow were seen, whereas islet blood flow was decreased after glipizide administration. Consequently, fractional islet blood was also diminished.

## DISCUSSION

$K_{ATP}$ channels are composed of a pore-forming unit that is a member of the inwardly rectifying $K^+$ channel family and constitutes either Kir6.1 or Kir6.2 (7). Normally, Kir6.1 is found in vascular smooth muscle cells (VSMCs), whereas Kir6.2 is located in β-cells (7). Recently Kir6.1 knockout mice were found to exhibit VSMCs, which have no detectable $K_{ATP}$ currents and lack all vasodilator responses to $K_{ATP}$ channel agonists (13). These animals showed spontaneous bouts of coronary vasospasm and ST-elevation, i.e., they reproduced key features of Prinzmetal angina (13). These findings would suggest that most VSMCs have Kir6.1 as their pore-forming unit. However, other studies have demonstrated that Kir6.2 also may be expressed in

TABLE 2
Measurements were made before (0 min) or 20 min after an intravenous injection of 2 ml/kg saline or diazoxide (10, 20, or 30 mg/kg) in anesthetized Sprague-Dawley rats

| Treatment | Treatment (dose) | | | |
|---|---|---|---|---|
| | Saline (0) | Diazoxide (10 mg/kg) | Diazoxide (20 mg/kg) | Diazoxide (30 mg/kg) |
| *n* | 7 | 8 | 8 | 8 |
| Duodenal blood flow ($ml \cdot min^{-1} \cdot g^{-1}$) | 1.56 ± 0.24 | 1.05 ± 0.26 | 1.96 ± 0.23* | 2.44 ± 0.45* |
| Colonic blood flow ($ml \cdot min^{-1} \cdot g^{-1}$) | 0.59 ± 0.20 | 0.55 ± 0.11 | 1.27 ± 0.25* | 1.06 ± 0.17* |
| Renal blood flow ($ml \cdot min^{-1} \cdot g^{-1}$) | 3.05 ± 0.43 | 3.49 ± 0.83 | 4.16 ± 0.59 | 3.67 ± 0.51 |
| Adrenal blood flow ($ml \cdot min^{-1} \cdot g^{-1}$) | 2.99 ± 0.17 | 3.52 ± 0.75 | 4.16 ± 0.59 | 3.67 ± 0.51 |

Data are means ± SE. *$P < 0.05$ compared with animals given diazoxide 10 mg/kg. All comparisons were made with ANOVA using Bonferroni's correction.

TABLE 3
Measurements were made 20 min after an intravenous injection of 2 ml/kg saline or diazoxide (10, 20, or 30 mg/kg) in anesthetized Sprague-Dawley rats

| Treatment | Treatment (dose) | | | |
|---|---|---|---|---|
| | Saline (0) | Diazoxide (10 mg/kg) | Diazoxide (20 kg/mg) | Diazoxide (30 mg/kg) |
| *n* | 7 | 8 | 8 | 8 |
| Pancreas ($m \cdot g^{-1} \cdot mmHg^{-1}$) | 5.7 ± 0.7 | 12.6 ± 1.9* | 26.5 ± 2.2†‡ | 30.0 ± 3.8†‡ |
| Duodenum ($m \cdot g^{-1} \cdot mmHg^{-1}$) | 17.1 ± 3.5 | 17.4 ± 5.0 | 42.9 ± 5.4†‡ | 60.1 ± 10.7†‡ |
| Colon ($m \cdot g^{-1} \cdot mmHg^{-1}$) | 6.3 ± 2.2 | 8.7 ± 1.6 | 26.9 ± 4.9*§ | 26.2 ± 4.3*† |
| Kidney ($m \cdot g^{-1} \cdot mmHg^{-1}$) | 33.5 ± 6.7 | 58.1 ± 14.3 | 67.8 ± 8.9* | 92.3 ± 11.1†‖ |
| Adrenals ($m \cdot g^{-1} \cdot mmHg^{-1}$) | 25.4 ± 4.2 | 58.8 ± 13.9 | 88.3 ± 11.4† | 91.0 ± 12.5† |

Data are means ± SE. *$P < 0.01$ and †$P < 0.001$ when compared with saline-injected rats; ‡$P < 0.001$, §$P < 0.01$, ‖$P < 0.05$ when compared with animals given diazoxide 10 mg/kg. All comparisons were made with ANOVA using Bonferroni's correction.

some muscle cells, and this confers different properties to VSMCs depending on whether their $K_{ATP}$ channels contain Kir6.1 or Kir6.2 (14).

The potassium channel also consists of a regulatory subunit, the sulfonylurea receptor (SUR). This part of the channel complex exists in three forms: SUR1 found in β-cells and some neurons, SUR2A in cardiac and skeletal muscle, and SUR2B in VSMCs (7). In confirmation of this, recent experiments on SUR2 knockout mice demonstrated findings similar to those seen in Kir6.1 knockouts (i.e., a Prinzmetal phenotype and loss of $K_{ATP}$ channels in VSMCs) (15).

The mechanism behind the blood flow increase presently seen after administration of diazoxide is likely to be an opening of VSMC $K_{ATP}$ channels in pancreatic resistance vessels, i.e., presumably mainly arterioles. This would lead to a hyperpolarization of the muscle cell causing relaxation and vasodilatation (6,16,17). However, diazoxide is not selective for VSMC or β-cell $K_{ATP}$ channels, but affects both. It should be kept in mind that there are no pharmacological or physiological studies that have determined the type of $K_{ATP}$ channels present in pancreatic vasculature.

It has been suggested that diazoxide is selective for mitochondrial $K_{ATP}$ channels in heart muscle (18–20). An effect on these channels has been suggested to mediate the protection conferred by this potassium channel opener against toxins in the heart (21,22) and the central nervous system (23–25). In β-cells, on the other hand, diazoxide has been suggested to act on $K_{ATP}$ channels in both plasma membrane (26) and mitochondria (27). Indeed, the selectivity of diazoxide for mitochondrial $K_{ATP}$ channels, irrespective of tissue, is likely to be dose dependent (28). The doses used in the present study are likely to affect channels on both of these cellular locations.

When interpreting the effects on blood flow by diazoxide, it should be noted that there was a marked decrease in mean arterial blood pressure at all doses given. Because organ blood flows other than those to the pancreas remained unchanged, the vascular conductivity was markedly increased in these organs. Such an increase was even more pronounced in the pancreas and presumably also in the islets, leading to an absolute increase in these organ blood flow values. The value for the islets is not included in the table, because we do not have any measurement of the weight of the islet organ, which precludes an exact calculation of vascular conductance. However, it can be estimated, assuming an islet volume of 1–2%, that it is increased to the same degree as that to the whole pancreas. Thus, it seems as if opening of the $K_{ATP}$ channels induces most pronounced blood flow effects on islets and whole pancreas, despite the low blood pressure, which, nevertheless, is within the limits of autoregulation for the rat pancreas (29) and islets (30). Diazoxide had its most pronounced effects on total pancreatic blood perfusion, as manifested by the decreased fractional islet blood flow (i.e., a lowering of the fraction of blood diverted through the islets). This would suggest that arterioles in the exocrine parenchyma have more $K_{ATP}$ channels or that the VSMCs in these arterioles are more sensitive to diazoxide. We would like to once again stress that there are no available anatomical or pharmacological studies on intrapancreatic $K_{ATP}$ channel distribution.

In contrast to diazoxide, the newly described potassium channel opener NNC 55-0118 stimulated total pancreatic and islet blood flow to the same degree. No effects on the other measured regional blood flow values were seen. This is somewhat surprising, because NNC 55-0118 is selective for the Kir6.2/SUR1 $K_{ATP}$ channel of the β-cell (11). Indeed, the effects on VMSCs are small and previous studies have demonstrated blood pressure effects similar to those in the present study (11). This opens the possibility that the

TABLE 4
Measurements were made before (0 min) or 20 min after an intravenous injection of vehicle or NNC 55-0118 (3 mg/kg) in anesthetized Sprague-Dawley rats

| | Treatment | |
|---|---|---|
| | Vehicle | NNC 55-0118 |
| *n* | 8 | 8 |
| Mean arterial blood pressure (mmHg) | 120 ± 6 | 98 ± 6* |
| Blood glucose (mmol/l) | | |
| 0 min | 7.6 ± 0.3 | 7.0 ± 0.4 |
| 20 min | 6.7 ± 1.0 | 7.4 ± 0.4 |
| Serum insulin (ng/ml) | 1.28 ± 0.31 | 0.56 ± 0.12* |
| Duodenal blood flow ($ml \cdot min^{-1} \cdot g^{-1}$) | 1.99 ± 0.29 | 1.91 ± 0.19 |
| Colonic blood flow ($ml \cdot min^{-1} \cdot g^{-1}$) | 0.41 ± 0.07 | 0.36 ± 0.14 |
| Renal blood flow ($ml \cdot min^{-1} \cdot g^{-1}$) | 2.19 ± 0.30 | 3.16 ± 0.39 |
| Adrenal blood flow ($ml \cdot min^{-1} \cdot g^{-1}$) | 2.64 ± 0.33 | 3.67 ± 0.57 |

Data are means ± SE. *$P < 0.01$ when compared with vehicle-treated rats. Comparisons were made with Student's unpaired *t* test.

TABLE 5
Measurements were made before (0 min) or 45 min after an intravenous injection of vehicle or glipizide (25 μg/kg) in anesthetised Sprague-Dawley rats

| | Treatment | |
|---|---|---|
| | Vehicle | Glipizide |
| *n* | 8 | 8 |
| Blood glucose (mmol/l) | | |
| 0 min | 4.7 ± 0.2 | 4.2 ± 0.1 |
| 45 min | 4.4 ± 0.1 | 2.4 ± 0.3*† |
| Total pancreatic blood flow ($ml \cdot min^{-1} \cdot g^{-1}$) | 0.57 ± 0.10 | 0.55 ± 0.05 |
| Islet blood flow ($\mu l \cdot mm^{-1} \cdot g$ $pancreas^{-1}$) | 57 ± 10 | 35 ± 3*† |
| Islet blood flow (% of pancreatic blood flow) | 9.0 ± 0.4 | 6.5 ± 0.7* |

Data are means ± SE. *$P < 0.05$, †$P < 0.01$ when compared with vehicle-treated rats. Comparisons were made with Student's unpaired *t* test.

effects of the $K_{ATP}$ channel openers on islet blood perfusion are not primarily dependent on activation of VMSCs potassium channels (i.e., those primarily containing Kir6.1/Sur2B potassium channels). It can instead be that activation of the β-cell Kir6.2/SUR1 channel and/or that substances released from the islet cells themselves affect the islet arteriolar blood vessels and lead to the marked increase in blood flow. This notion would then explain why diazoxide increases the vascular conductance more in the pancreas and islets, since diazoxide would also affect the common Kir6.1-containing VSMC potassium channels, leading to the general increase in vascular conductance seen in all studied organs, including the whole pancreas. Furthermore, diazoxide would also affect the β-cell $K_{ATP}$ channel, which could cause a further increase in islet blood flow (see below) and, perhaps, total pancreatic blood flow. Several previous studies have demonstrated the independent regulation of islet blood flow when compared with the exocrine pancreas (1,2).

In favor of such an interpretation is our finding after administration of glipizide, i.e., a closer of $K_{ATP}$ channels, with a selectivity for the β-cell Kir6.2/SUR1 form. Glipizide leads to a decrease in islet blood flow but had no effects on total pancreatic blood flow. In line with the anatomical organization of islet afferent blood supply (see above), it can also be that a substance affecting the islets may release substances that can diffuse to the islet arterioles and directly affect VSMCs. Immunolocalization of $K_{ATP}$ channels in rat islets have demonstrated that SUR1 and Kir6.2 are found not only in β-cells, but also in α-, δ-, and pancreatic polypeptide cells (31). This implies that any of these endocrine cells can be a source of a substance(s) that may affect the islet afferent arteriole. At present, the nature of such a mediator is unknown. Glucagon is worthy of mentioning in this context because it is a vasoactive hormone (32). Furthermore, the administered glipizide caused hypoglycemia, which is likely to have increased glucagon release. Preliminary findings with insulin-induced hypoglycemia also demonstrate a decreased pancreatic islet blood flow, and this response seems to be nervously mediated (P.-O. Carlsson, L.J., unpublished observations). Thus, to what extent the decreased islet blood flow seen after glipizide administration is due to an effect on $K_{ATP}$ channels or is nervously mediated is at present unknown.

A piece of indirect evidence pointing toward an effect of $K_{ATP}$ channels in the regulation of normal islet blood flow is the association between these potassium channels and adenosine. We have recently shown that adenosine, probably produced by β-cells, can stimulate islet blood flow during conditions associated with increased islet metabolism (3). Moreover, a coupling between $K_{ATP}$ channels and adenosine effects on pancreatic vasculature has been suggested in experiments on the perfused rat pancreas (33). In this context, it has been reported that heart microvascular dilation in response to adenosine is enhanced at lower intraluminal pressures by selective activation of VSMC $K_{ATP}$ channels (5). This should be compared with islets, where the capillary blood pressure is lower than in the exocrine parenchyma, but where the blood flow response to adenosine is more pronounced (3).

To what extent the increased blood glucose concentrations may affect the islet blood perfusion in the present study is of interest. It has previously been demonstrated that hyperglycemia may increase the blood perfusion of the islets (34), presumably mediated by nitric oxide (35). Diazoxide increased the values to ~9 mmol/l, whereas NNC 55-0118 had no such effects. In view of the similarity on blood flow response but differences in blood glucose concentrations, it seems unlikely that the latter is responsible for the changes in islet blood flow. Furthermore, in a previous study, we observed that the blood glucose concentrations needed for this response to occur in anesthetized animals were higher than those seen in the present study (34). In general, however, the blood flow changes seen in the present study include effects mediated by $K_{ATP}$ channels as well as more unspecific changes induced by altered blood pressure, blood glucose (see above), and serum insulin concentrations.

In an earlier study, the sulfonylurea tolbutamide was found to increase blood flow to the islets in rats in doses inducing hypoglycemia, but had no effects on total pancreatic blood flow (36). The reasons for the discrepancy between their findings and those in the present study are unknown. In the tolbutamide study, young rats, aged 5 weeks, were used, whereas we studied adult rats ~15 weeks old. We have previously noted marked differences in the absolute values in islet blood flow depending on the age of the animals, and it is also likely that the blood flow regulation differs (37,38). It is also possible that there are differences in the actions of tolbutamide and glipizide, although this seems less likely. However, in view of previous findings when applying sulfonylureas, we consider a decrease in islet blood flow to be a logical consequence of administering these substances. Thus, depending on the dose, glibenclamide is known to reduce early and peak vasodilatation following reactive hyperemia in humans (39). Furthermore, glibenclamide (20 mg/kg) to rats increased vascular resistance and caused a systemic vasoconstriction (40). Therefore, it seems as if $K_{ATP}$ channel closers would favor a vasoconstriction rather than a dilation. It may seem physiologically appropriate that insulin release induced by sulfonylureas would be associated with an increased blood perfusion, as suggested by Vetterlein et al. (36). However, in other contexts, we have been able to

show that the very high basal blood perfusion of the islets allows for a substantial reduction in islet blood flow before any effects on insulin secretion can be observed (1).

In summary, our results suggest that $K_{ATP}$ channels play an important role for regulation of total pancreatic and islet blood flow. To what extent effects on islet function are mediated by a modulated islet blood perfusion by substances affecting these channels merits further investigation.

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