Partial pancreatectomy in rats causes an impairment of the glucose-induced stimulation of pancreatic islet blood flow

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Summary. Adult rats were subjected to either a sham operation (S-rats) or a 60% partial pancreatectomy (P-rats). Both P- and S-rats were normoglycemic and normoinsulinemic after surgery. Four weeks later, the animals were injected i.v. with 1 ml of either 0.9% (w/v) saline or 30% (w/v) D-glucose, and after 5 min whole pancreatic blood flow (PBF) and islet blood flow (IBF) were measured, using a microsphere technique. In the saline-injected P-rats both PBF and IBF values were higher than in S-rats ($p < 0.001$ for both values). Administration of glucose had no effects on PBF in either S- or P-rats when compared to saline-injected animals. IBF was, however, markedly increased ($p < 0.01$) by glucose in S-rats in comparison with saline-injected S-rats, whilst no difference in IBF was observed between glucose- and saline-injected P-rats. The fraction of PBF diverted through the islets (fIBF) was approximately 10% in S-rats and 20% in P-rats. Glucose increased fIBF in S-rats, but had no effect in P-rats. In conclusion, in S-rats a glucose-stimulated insulin release is accompanied by an increase in IBF, but this is not observed in P-rats.
Key words. Pancreatic islets; islet blood flow; partial pancreatectomy.

Partial pancreatectomy imposes increased functional demands on the remaining endocrine and exocrine tissues of the gland. On the one hand, the tissues must immediately compensate for the injury by an increased function of the remaining cells. On the other hand, an initiation of growth to replace the removed cells must take place. It is well known that the endocrine capabilities of the pancreatic islets are sufficient to cope with a severe reduction in the number, usually exceeding 90%, of functioning B-cells, before any abnormalities of glucose homeostasis occur[1, 2]. It has previously been demonstrated that a 60% partial pancreatectomy caused an increased growth of the gland but produced no changes in the glucose tolerance of adult rats[3]. One marked effect of the partial pancreatectomy was a pronounced but transient increase in the whole pancreatic blood flow (PBF), and an even more marked and also permanent increase in islet blood flow (IBF), with peak flow values observed 4 weeks after surgery[3]. At this time, the IBF was approximately 4 times the basal blood perfusion observed in sham-operated rats. In another study, the model with partially pancreatectomized rats was used in combination with islets grafted under the renal capsule, and similar values of both IBF and PBF were observed[4]. However, glucose, which normally stimulates IBF[5], had no effect on IBF in these rats[4]. One confounding factor in these experiments was the presence of the islet graft, consisting of 500 islets under the renal capsule, with an unknown effect on the blood perfusion of the native pancreas. The aim of the present study was to perform blood flow measurements in partially pancreatectomized and sham-operated rats 4 weeks after surgery, to evaluate whether glucose can affect the pancreatic blood perfusion in these animals, as it does in normal ones.

Materials and methods

Animals. Male Sprague-Dawley rats weighing approximately 350 g were obtained from a local breeding colony at the Biomedical Center, Uppsala, Sweden. The animals had free access to tap water and pelleted food (Type R3; Ewos, Södertälje, Sweden) and were housed two per cage in rooms with constant temperature (22 °C) and humidity (70%) and a 12-h light/dark cycle.

Surgical treatment: The animals were pre-treated with atropine sulphate (Atropin®; 0.05 mg/kg b.wt i.p.; ACO Läkemedel, Stockholm, Sweden) and then anesthetized with an intraperitoneal injection of sodium pentobarbital (Mebumal®; 40 mg/kg b.wt; ACO Läkemedel), which was supplemented, if necessary, with ether during the operation. After induction of anesthesia, the splenic 60% of the pancreas (approximately 1 g) was removed (P-rats) as previously described[3]. In sham-operated animals (S-rats) the pancreas was handled to the same extent, but without damaging or removing any pancreatic tissue. Two rats of the same age and housed in the same cage were operated on on the same day, one partially depancreatized and one sham-operated, and they were also housed in the same cage after surgery.

Blood flow measurements. Four weeks after the partial pancreatectomy or sham-operation the blood perfusion of the whole pancreas and the islets was measured with a microsphere technique as previously described in detail[3]. Briefly, the animals were anesthetized with thiobutabarbital (Inactin®; 120 mg/kg b.wt i.p.; Byk Gulden Konstanz, FRG) and heparinized. Under control of mean arterial blood pressure and body temperature, polyethylene catheters were inserted into the ascending aorta, through the right carotid artery, and into the lower part of the abdominal aorta. Five minutes before the

blood flow measurements the animals were injected intravenously with 1 ml of either 0.9% (w/v) saline or 30% (w/v) D-glucose. Nonradioactive microspheres (New England Nuclear, Boston, MA, USA) with a diameter of approximately 10 μm were then injected ($\approx 1-1.5 \times 10^5$ microspheres) through the catheter with its tip in the ascending aorta. Simultaneously an arterial blood reference sample was withdrawn from the other arterial catheter at a rate of approximately 0.60 ml/min. The exact withdrawal rate was confirmed in each experiment by weighing the sample. Immediately after the reference sample had been secured, arterial blood was collected and later analyzed for glucose concentration with an automated glucose oxidase technique (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA, USA) and for insulin concentration with radioimmunoassay[6]. The pancreas and the adrenal glands were then removed, blotted and weighed before being treated for visualization of islets and microspheres by a freeze-thawing technique[7].

The number of microspheres in the whole pancreas, the islets, the adrenal glands and the blood reference sample was counted as previously described[3]. The blood perfusion of the pancreas and the islets could then be calculated according to the formula: $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$, where Q_{org} = organ blood flow (ml/min), Q_{ref} = withdrawal rate of the reference sample (ml/min), N_{org} = the number of microspheres present in the organ, N_{ref} = the number of microspheres present in the reference sample. The blood flow values calculated for the paired adrenal glands were used to confirm that the microspheres were mixed adequately with the circulation. A difference in blood flow values of less than 10% was taken to indicate sufficient mixing of the microspheres.

Statistical calculations. All values are given as means ± SEM. Probabilities (P) of chance differences between the experimental groups were calculated by ANOVA.

Results

A total of three animals died owing to complications after surgery. Three further animals were excluded from the study since the microsphere content of their adrenal glands exceeded 10%, suggesting an inadequate mixing of the microspheres. Four weeks after surgery the pancreatic glands were smaller in the animals subjected to the 60% partial pancreatectomy (986 ± 120 mg in P-rats and 1576 ± 87 mg in S-rats respectively; $p < 0.001$). All animals remained normoglycemic after the sham-operation or partial pancreatectomy (data not shown), and had normal serum glucose and serum insulin concentrations at the time of the blood flow measurements (table 1). Glucose administration increased serum glucose and serum insulin to the same degree in S- and P-rats (table 1). The mean arterial blood pressure was also similar in all animals (data not shown).

The whole pancreatic blood flow (PBF) and the islet blood flow (IBF) was markedly higher in the P-rats compared to S-rats (table 2). The fractional IBF (fIBF; i.e. the fraction of PBF diverted through the islets) was approximately 10% in S-rats, but 20% in the P-rats (table 2). Injection of glucose caused no change in PBF in either P- or S-rats, whilst both IBF and fIBF was increased in S-rats, but not in P-rats (table 2).

Discussion

The 60% partial pancreatectomy had no effect on the serum glucose or serum insulin concentrations, confirm-

Table 1. Serum glucose and serum insulin concentrations in 2/3 partially pancreatectomized (P-rats) and sham-operated rats (S-rats) 5 min after an intravenous injection of 1 ml of either 0.9% (w/v) saline or 30% (w/v) D-glucose.

Group of animals	Substance injected	Serum glucose concentration (mmol/l)	Serum insulin concentration (ng/ml)
S-rats (7)	Saline	8.7 ± 0.6	1.89 ± 0.26
S-rats (6)	Glucose	22.3 ± 2.9*	4.57 ± 0.58*
P-rats (8)	Saline	8.4 ± 0.7	1.51 ± 0.20
P-rats (10)	Glucose	23.9 ± 3.0	4.17 ± 0.25**

All values are given as means ± SEM. The number of experiments in each group is given within parentheses. *denotes $p < 0.001$ compared to saline-injected S-rats, and **denotes $p < 0.001$ compared to saline-injected P-rats.

Table 2. Whole pancreatic blood flow (PBF), islet blood flow (IBF) and islet blood flow expressed as a fraction of PBF (fIBF) in 2/3 partially pancreatectomized (P-rats) or sham-operated rats (S-rats) 5 min after an intravenous injection of 1 ml of either 0.9% (w/v) saline or 30% (w/v) D-glucose.

Group	Substance injected	PBF (ml/min × g)	IBF (μl/min × g)	fIBF (% of PBF)
S-rats (7)	Saline	0.58 ± 0.08	62 ± 11	10.4 ± 1.1
S-rats (6)	Glucose	0.65 ± 0.09	87 ± 5**	14.8 ± 1.0**
P-rats (8)	Saline	1.08 ± 0.13***	223 ± 31***	20.5 ± 1.4***
P-rats (10)	Glucose	1.03 ± 0.07	202 ± 30	19.5 ± 2.6

All values are given as means ± SEM. The number of experiments in each group is given within parentheses. **denotes $p < 0.01$ and ***denotes $p < 0.001$ compared to saline-injected S-rats.

ing previous observations[3,8]. There was a marked increase in weight of the pancreatic remnant in P-rats, as evidenced by the weight of the pancreas in P-rats which was approximately 60% of that of S-rats at the end of the experimental period although 60% of the whole gland had been removed 4 weeks earlier. This increase is, according to previous observations, more marked for the endocrine component[9], and is due to both hyperplasia and hypertrophy[10].

PBF was markedly increased in P-rats when compared to S-rats[3,4], which probably reflects the increased functional demands on the pancreatic remnant caused by the partial pancreatectomy[3]. IBF and fIBF were also increased in P-rats, but whilst administration of glucose caused the expected preferential increase in IBF and fIBF in S-rats[11], it had no effect on the flow values of the P-rats. It should be noted in this context that the serum insulin concentrations were similar in S- and P-rats after glucose administration. This suggests that an increased insulin secretion occurred without a simultaneous increase in IBF in the P-rats, which is in contrast to the findings in the S-rats, where IBF increased. The reasons for this discrepancy are unknown. It may, however, be a result of the fact that the islets in P-rats are exposed to high functional demands with an associated increase in cell metabolism. Factors released from these metabolically active cells might directly stimulate the IBF, in analogy to what happens in the intestine[12,13]. If the IBF is already stimulated, the expected 50% increase in IBF caused by glucose[11], would not be noticeable. The nature of these locally active factors is conjectural, but they might be substances affecting purinergic receptors, e.g. adenosine which is known to be released from B-cells[14]. The increased pancreatic blood perfusion in P-rats may also be associated with the stimulation of growth of the islet organ caused by the partial pancreatectomy. This is indicated by our previous findings referred to above in rats with a graft consisting of 500 islets, that is a transplant insufficient to cure diabetes[4], but nevertheless containing approximately 10% of the number of islets normally found in a rat pancreas[7]. These animals, with a 'reserve supply' of islet tissue, and therefore smaller functional demands of the islets in the native pancreas, also had markedly increased IBF and PBF values in the native pancreas, which failed to be influenced by glucose. This observation, in combination with the present findings, suggests that the functional activity of the islet cells is not a single causative factor for the increased blood perfusion observed after partial pancreatectomy. The combined data from the previous work[4], and the present findings, suggest that it is the local environment within the growing pancreas that causes the inability of glucose to stimulate the IBF. The nature of these environmental factors is presently unknown.

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