Duct ligation and pancreatic islet blood flow in rats: physiological growth of islets does not affect islet blood perfusion

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Abstract
Objectives: The aim of this study was to evaluate islet blood-flow changes during stimulated growth of the islet organ without any associated functional impairment of islet function.
Design: A duct ligation encompassing the distal two-thirds of the pancreas was performed in adult, male Sprague–Dawley rats.
Methods: Pancreatic islet blood flow was measured in duct-ligated and sham-operated rats 1, 2 or 4 weeks after surgery. In some animals studied 4 weeks after surgery, islet blood flow was also measured also during hyperglycaemic conditions.
Results: A marked atrophy of the exocrine pancreas was seen in all duct-ligated rats. Blood glucose and serum insulin concentrations were normal. An increased islet mass was only seen 4 weeks after surgery. No differences in islet blood perfusion were noted at any time point after duct ligation. In both sham-operated and duct-ligated rats islet blood flow was increased during hyperglycaemia; the response was, however, slightly more pronounced in the duct-ligated part of the gland.
Conclusions: Normal, physiological islet growth does not cause any major changes in the islet blood perfusion or its regulation. This is in contrast to findings during increased functional demands on the islets or during deteriorated islet function, when increased islet blood flow is consistently seen.

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Introduction
The vasculature of the pancreatic islets is autonomous, both anatomically and physiologically, from that of the exocrine parts of the gland (1, 2). The regulation of islet blood perfusion is exerted via complex interactions between nervous, endocrine, paracrine and metabolic signals to maintain a high blood flow, adapted to the immediate needs of the endocrine cells (2, 3). Studies have shown that conditions inducing increased functional demands on the islets are associated with increases in islet blood perfusion (1). If, and to what extent, this blood flow increase affects islet function is unknown. We have, however, observed an increased capillary blood pressure in the islets of GK rats (4), a model of type 2 diabetes. In view of the hemodynamic hypothesis for development of diabetes complications (5), an increased shear stress on endothelial cells may affect and alter their expression of paracrine substances, which may then also influence more specific organ functions. A recent study on pregnant rats, where the increased demands of insulin are met by growth of the islets, showed that even if total islet blood flow was increased, islet blood perfusion was actually decreased when corrected for islet mass (6). The major source of new β-cells during pregnancy is the division of pre-existing islet cells translating into islet hyperplasia (7). The growth is likely to be associated with an accompanying replication in the intra-islet vasculature with an increased demand for islet blood perfusion. Islet neogenesis also produces an increase in β-cell mass, mainly from precursor cells in the ductal system (8). One way to experimentally stimulate such a neoformation of islets is partial duct ligation (9, 10). The aim of the present study was to continue our evaluation of islet blood-flow changes during stimulated growth of the islet organ. We especially wanted to establish whether increased islet blood perfusion could exist without any functional impairment of islet function. We therefore investigated whether islet growth induced by partial (50%) duct ligation in the rat, which is not associated with any impairment in glucose tolerance, led to any changes in islet blood perfusion. Thus, if a hyperperfusion of
blood through the islets was seen in this model, it would be unlikely that increased islet blood by itself, in the absence of inherent β-cell abnormalities, could adversely affect islet endocrine function.

Materials and methods

Experimental animals
Male Sprague–Dawley rats, aged 10–12 weeks, were purchased from B&K Universal (Sollentuna, Sweden) and subsequently used in all experiments. All animals had free access to tap water and pelleted rat food throughout the experiments. All experiments were approved by the local animal ethics committee at Uppsala University, and were conducted in accordance with accepted standards of humane animal care.

Surgical procedures

The animals were anaesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg body weight; Apoteksbolaget, Umeå, Sweden), and placed on a heated operating table. A 2 cm-long midline abdominal incision was made and the pancreatic ducts of the corpus and cauda of the pancreas were identified (8, 9). In some animals a silk ligature was placed around these ducts, at the junction between the corpus and caput regions and ligated. In sham-operated controls the pancreas was handled to the same extent without interfering with the drainage of the ducts. The rats were then allowed to recover from anaesthesia, and were kept single in cages for the remainder of the experiment.

Blood-flow measurements

The rats were anaesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg/kg body weight; Inactin; Research Biochemicals International, Natick, MA, USA) 1, 2 or 4 weeks after sham surgery, or partial duct ligation. The rats were then placed on a heated operating table to maintain body temperature at approximately 38°C. 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, Groby, Leicestershire, UK), whereas the latter was used to infuse Ringer solution (5 ml/kg body weight per h) to substitute for fluid losses. When the blood pressure had remained stable for at least 20 min, blood-flow measurements were performed with a microsphere technique as described previously (8, 9, 10). Briefly, a total of (1.5-2.0) x 10^5 black non-radioactive microspheres (EZ-Trac™; Triton Microspheres, San Diego, CA, USA), with a diameter of 10 μm, were injected via the catheter with its tip in the ascending aorta over a period of 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 approximately 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. Some of the animals studied 4 weeks after surgery were injected intravenously with 1 ml of a 30% (w/v) D-glucose solution. A new microsphere injection was performed 3 min later, using blue instead of black microspheres (11). Thus, after one (1 or 2 weeks after surgery) or two (4 weeks after surgery) microsphere injections the animals were killed, and the pancreas and adrenal glands were removed. The pancreas was divided into two sections, corresponding to the caput and corpus + cauda, respectively, and each section treated separately. The caput and cauda regions are the parts encompassed by the duct ligation, whereas the caput was unaffected in all animals. After weighing, the different parts of the pancreata were treated with a freeze–thawing technique, which visualized the pancreatic islets and microspheres (11, 13). The number of microspheres of each color in these samples was then counted in a microscope equipped with both bright- and dark-field illumination. The visualization of the islets in these samples enabled us to estimate the volume of the islets within the pancreatic regions, since islets with a diameter exceeding 50 μm can be identified, and thereby also the islet mass by a point-counting method described in detail previously (14, 15). The number of microspheres in the arterial reference sample was determined by sonicating the blood, transferring samples to glass microfibre filters (pore size < 0.2 μm), and counting them under a microscope.

The organ 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. Islet blood perfusion was expressed in terms of both g wet weight of the whole pancreas, and the estimated wet weight of the islets themselves. Blood-flow values based on the microsphere contents of the adrenal glands were used to confirm that the microspheres were mixed adequately in the circulation. A difference of less than 10% in the blood-flow values was taken to indicate sufficient mixing, which occurred in all animals in the present study (results not shown).

Morphological examination

Some sham-operated or duct-ligated rats were not used for blood-flow measurements, but killed by an overdose of thiobutabarbitral (Sigma) 1, 2 or 4 weeks after surgery, and the pancreas removed as described above.
The pancreatic specimens were then fixed in formalin, embedded in paraffin, sectioned and stained with van Gieson’s stain.

**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 ELISA (Rat Insulin ELISA; Mercodia AB, Uppsala, Sweden) with rat insulin as a standard.

**Statistical calculations**

All values are given as means ± S.E.M. Probabilities (P) of chance differences were calculated with Student’s paired or unpaired t-test, or with one-way, repeated-measurement analysis of variance (ANOVA) with Bonferroni’s correction (SigmaStat; SSPD, Erfurt, Germany). A value of P < 0.05 was considered to be statistically significant.

**Results**

**General status of the animals**

All animals tolerated the sham operation or duct-ligation surgery without any signs of infirmity, ate normally and resumed a normal body-weight gain (results not shown). At the time of the blood-flow measurements body weights, blood glucose and serum insulin concentrations did not differ between the groups (Table 1). Mean arterial blood pressure (Table 1) and hematocrit (results not shown) were also similar in all groups.

**Table 1** Body weight, mean arterial blood pressure, blood glucose and serum insulin concentrations in Sprague–Dawley rats subjected to sham operation (Sham) or partial duct ligation (DL) 1, 2 or 4 weeks earlier.

<table>
<thead>
<tr>
<th>Surgical treatment</th>
<th>Sham</th>
<th>DL</th>
<th>Sham</th>
<th>DL</th>
<th>Sham</th>
<th>DL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time after surgery (weeks)</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>No. of animals</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>357±7</td>
<td>345±10</td>
<td>321±5</td>
<td>338±17</td>
<td>455±9</td>
<td>444±8</td>
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<tr>
<td>Pancreas weight (mg)</td>
<td>538±30</td>
<td>591±25</td>
<td>422±30</td>
<td>474±55</td>
<td>719±28</td>
<td>767±17</td>
</tr>
<tr>
<td>Head</td>
<td>570±43</td>
<td>254±40*</td>
<td>405±24</td>
<td>104±18*</td>
<td>719±34</td>
<td>212±34*</td>
</tr>
<tr>
<td>Body + tail</td>
<td>0.90±0.03</td>
<td>0.85±0.06</td>
<td>0.82±0.09</td>
<td>0.83±0.08</td>
<td>0.90±0.05</td>
<td>0.94±0.07</td>
</tr>
<tr>
<td>Islet volume (% of pancreas)</td>
<td>0.92±0.04</td>
<td>2.24±0.20*</td>
<td>1.11±0.10</td>
<td>5.19±1.16†</td>
<td>1.01±0.05</td>
<td>6.08±1.212†</td>
</tr>
<tr>
<td>Head</td>
<td>4.85±0.31</td>
<td>5.00±0.38</td>
<td>3.48±0.49</td>
<td>4.06±0.82</td>
<td>6.51±0.49</td>
<td>7.17±0.52</td>
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<tr>
<td>Body + tail</td>
<td>5.22±0.48</td>
<td>5.57±0.83</td>
<td>4.54±0.50</td>
<td>4.71±0.55</td>
<td>7.23±0.30</td>
<td>10.27±0.81†</td>
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<tr>
<td>Blood glucose (mM)</td>
<td>6.7±0.2</td>
<td>7.1±0.04</td>
<td>6.1±0.2</td>
<td>6.2±0.3</td>
<td>7.7±0.2</td>
<td>7.6±0.2</td>
</tr>
<tr>
<td>Serum insulin (ng/ml)</td>
<td>3.08±0.67</td>
<td>4.01±1.02</td>
<td>3.60±0.47</td>
<td>2.40±0.50</td>
<td>4.08±0.70</td>
<td>3.86±0.48</td>
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<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>106±7</td>
<td>110±8</td>
<td>115±11</td>
<td>113±6</td>
<td>111±10</td>
<td>112±9</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. *P < 0.01 when compared to the corresponding value in the head region of the same animal (Student’s paired t-test). †‡P < 0.05 when compared to the corresponding value in duct-ligated rats 1 week after surgery (one-way, repeated-measurement ANOVA with Bonferroni’s correction).

**Pancreatic morphology**

Visual inspection of the pancreas of the partially duct-ligated rats in a low-power microscope demonstrated a marked atrophy of the exocrine part of the ligated gland. Furthermore, numerous islets could be seen in these regions. The identity of the islets was confirmed by conventional histological sections (results not shown). The atrophy was also manifested by a markedly lower weight of the ligated part of the pancreas when compared with the corresponding regions of the sham-operated rats (Table 1). Due to the atrophy of the exocrine pancreas the relative islet volume was increased markedly in the ligated part of the gland at all time points evaluated, with the highest values seen 4 weeks after surgery (Table 1). However, the absolute islet mass in the duct-ligated pancreas increased only after 4 weeks (Table 1).

**Pancreatic and islet blood flow**

Total pancreatic blood flow was similar in all regions of the pancreas in sham-operated and duct-ligated rats (Fig. 1). Due to the atrophy of the exocrine pancreas and relative increase in islet volume islet blood flow was higher at all time points in the duct-ligated parts of the pancreas when expressed in terms of pancreas weight (Fig. 2). This increase was most pronounced 4 weeks after surgery (Fig. 2). However, there were no differences between sham-operated and duct-ligated rats at any of the studied time points when islet blood perfusion was corrected for islet mass (Fig. 3A).

**Blood flow during hyperglycaemia**

When glucose was administered to sham-operated or partially duct-ligated rats 4 weeks after surgery blood
glucose and serum insulin concentrations increased as expected (Table 2). Mean arterial blood pressure (Table 2), hematocrit (results not shown) and total pancreatic blood flow were unaffected by glucose administration (Table 2). Islet blood perfusion expressed in terms of islet weight was increased to the same extent in the caput and corpus + cauda regions of both sham-operated and partially duct-ligated rats (Fig. 3B). However, the glucose-induced increase in islet blood perfusion was more pronounced in the duct-ligated portion of the pancreas than in the non-ligated part (Fig. 3B).

Discussion

Partial duct ligation induced exocrine atrophy and expansion of islet mass, thereby confirming several previous observations (8–10). In rats there were a maximum number of TUNEL (terminal deoxynucleotidyl transferasemediated dUTP nickend labelling)-positive acinar cells on day 2 after duct ligation, with a return towards basal levels after 5 days (16). This time schedule of exocrine cell disappearance meant that the present experiments were performed well after the necrosis and atrophy had taken place.

Blood glucose and serum insulin concentrations were not affected by the surgical manipulations. We have also shown that islets isolated from duct-ligated rats functioned normally in vitro (17). Thus, no effect...
on the islet blood perfusion mediated by impaired carbohydrate metabolism was likely to occur in the present model.

Previous studies have demonstrated a doubling of β-cells and an increase in α-cells 1 week after surgery in rats (8). The findings were interpreted to reflect a neoinformation of islets from the ducts (8). Recent studies have shown an increased number of endocrine cells budding from the ducts during the first post-operative week in rats (18) and mice (19). Furthermore, cultured human ducts have been shown to form β-cells either in vitro (20), or after transplantation (21) and it is likely that most of the expansion of the islet mass seen in the present experiments was due to neoinformation, even though the study did not specifically address this question.

The mediation of the islet neoformation is likely to be multifactorial, and may even involve transdifferentiation of exocrine cells into insulin-producing β-cells (22). Proliferation and differentiation of ductal epithelium involve several growth factors (18, 23–25), which are likely to exert both endocrine and paracrine effects. To what extent such substances may affect local blood flow in the newly formed islets is at present unknown.

Blood perfusion of islets in the non-atrophied part of the gland was unaffected by the duct ligation. The islet blood perfusion expressed per pancreas weight or as a fraction of total pancreatic blood flow was very high at all time points after duct ligation. However, when islet blood flow was expressed per islet weight no difference in islet blood perfusion in any of the pancreatic regions for any time point in any of the groups was evident. The functional implications of this are not yet clear. It can be speculated that an augmented blood perfusion affects microvascular endothelium through increased shear stress, which in turn is thought to change the production of endothelial substances, exerting a paracrine effect on parenchymal cells, as occurs in other organs (4). An argument in favour of such a hypothesis is that the formation of new islets during pregnancy and neogenesis from ducts – physiologically ‘normal’ situations – are not associated with increased blood perfusion. Type 2 diabetes syndromes in rodents, on the other hand, are always associated with an increased islet blood flow (26), and also with capillary hypertension (27). The question as to why partially pancreatectomized rats do not develop type 2 diabetes, where the islet blood flow is increased, then arises. However, attention has focused recently on the importance of minute increases in blood glucose concentrations for the long-term effects on islet endocrine function during a β-cell mass decrease induced by partial pancreatectomy (12). Since we know from previous studies that islet blood flow is increased during these conditions (28), it may be that some of the deleterious effects of the increased blood glucose concentrations may be modulated and augmented by this hyperperfusion of blood. It is also very likely that a genetic component is involved in the sensitivity to this mechanism, which remains open to speculation.

The present study performed islet blood-flow measurements after glucose administration in the group of animals studied 4 weeks after surgery; that is, at a time point when there was an increase in islet mass. In accordance with previous observations (29, 30) acute hyperglycaemia induced a preferential increase in islet blood perfusion in both regions of the pancreas in sham-operated rats. This response was even more pronounced in the duct-ligated rat, suggesting that islet growth did not interfere with this important physiological response. This is in marked contrast to the findings after partial pancreatectomy, where no change in islet blood flow was seen during acute hyperglycaemia (3, 29), or in animal models of type 2 diabetes where islet blood perfusion was unaffected (31). Induced hyperglycaemia during pregnancy increases islet blood flow to a similar degree as in control animals (AM Svensson & L Jansson, unpublished observation). This once again underlines the fact that normal, physiological islet growth did not cause any apparent changes in the islet blood flow.
perfusion or its regulation, whereas increased functional demands, which may lead to deteriorated islet function, do.

The reasons for the more pronounced islet blood-flow increase in the duct-ligated part of the gland during hyperglycaemia are at present unknown. The early hyperglycaemia-induced islet blood-flow increase is mediated nervously (32), mainly through cholinergic innervation, and it has been suggested that there was a reduction in total ganglion cell number in duct-ligated pancreas, mainly those containing acetyl cholinesterase (19). Thus, a tentative, but speculative, explanation for the increased islet blood flow may be that a decrease in innervation may lead to hypersensitivity to cholinergic transmitters.

The blood supply of the ducts seems to be species-dependent (19). In mice and rabbits, which are the best-studied species, there was a common blood supply to ducts and ductal islets (11, 33). The newly formed islets, during islet neogenesis, receive both their afferent and efferent blood vessels from the ductal circulation. This vascular organization should be kept in mind when interpreting the islet blood-flow measurements performed with microspheres in the present study. A prerequisite for using this technique is that all microspheres became entrapped during their first passage through microvessels (34). This means that in serially connected capillary networks all microspheres were extracted in the first capillary system, and that none should pass into the second. It could thus be argued that if the newly formed ductal islets derive their vasculature from the ductal circulation, and that if this were in series with other capillary networks, the results obtained could be an underestimation of the islet blood perfusion. However, during the counting of the microspheres in the islets, microspheres in islets clearly adjacent to ducts were a consistent additional finding, so that the authors do not consider this to be a methodological problem.

Total pancreatic blood flow was unaffected in the caput and corpus + cauda regions of the pancreas in both sham-operated and duct-ligated rats. Thus, the exocrine atrophy did not affect the blood perfusion of the whole gland. This is somewhat surprising since chronic pancreatitis, another condition with exocrine atrophy, is usually associated with reduced pancreatic blood perfusion. It should be noted that islet blood flow in rats was approximately 10 times higher than total pancreatic blood flow, and that the islet volume increases more than 5-fold in the ligated parts of the gland, which obviously compensated for this decrease.

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