The use of hydrogen gas clearance for blood flow measurements in single endogenous and transplanted pancreatic islets

Andreea Barbu, Leif Jansson, Monica Sandberg, My Quach and Fredrik Palm

Original Publication:
http://dx.doi.org/10.1016/j.mvr.2014.10.002
Copyright: Elsevier
http://www.elsevier.com/
Postprint available at: Linköping University Electronic Press
http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-113736
The use of hydrogen gas clearance for blood flow measurements in single endogenous and transplanted pancreatic islets

Andreea Barbu a,b,⁎, Leif Jansson a,1, Monica Sandberg a, My Quach a, Fredrik Palm a,c

⁎ Corresponding author at: Department of Immunology, Genetics and Pathology, Uppsala University, Sweden
1 These authors contributed equally to this work.

A R T I C L E   I N F O

Article history:
Accepted 15 October 2014
Available online 23 October 2014

Keywords:
Blood flow
Hydrogen gas washout
Pancreatic islets
Transplanted islets

I N T R O D U C T I O N

Pancreatic islet blood perfusion has attracted increasing attention during the last decades (Jansson and Carlsson, 2002; Lammert, 2008). Indeed, islet vasculature has been shown to possess morphological and functional qualities making it uniquely adapted to the functional needs of this complex endocrine organ. Furthermore, it plays key roles in the development and growth as well as immunological and inflammatory reactions within the islets (Johansson et al., 2006a; Olerud et al., 2011). An obvious function is of course the transport of oxygen and nutrients to the islets, by means of blood perfusion. One serious problem for studies of islet blood flow has been the lack of suitable techniques to address this issue. The microsphere technique has been considered for decades to be the “gold-standard” for blood flow measurements in general (Prinzen and Bassingthwaighte, 2000) and its limitations and advantages when applied to islet blood flow have been discussed in detail (Jansson and Hellerström, 1983; Liason et al., 1980). Studies of whole islet organ blood perfusion with microspheres are undisputed, but there is an uncertainty regarding the reliability of this technique on the single islet level due to the very low blood perfusion of individual islets, amounting to only 20–50 nl mm−1 (Liason et al., 1980). This is especially so since the accuracy of the microsphere technique is based on the number of microspheres being present in the samples, in this case the islets. There is a limit to the number of microspheres that can be given to an animal due to induced circulatory alterations. This means that the blood flow represented by one microsphere will be similar to the single islet blood flow referred to above. Thus, some islets with that flow value will nevertheless not contain any microspheres. However, when all islets in a rat pancreas are considered as one organ, the flow value becomes more reliable, and the total number of islet microspheres counted is in the order of 400–500.

In the present study we have overcome these difficulties by adapting an old technique for blood flow measurements, namely the hydrogen gas washout technique, which has previously been extensively evaluated for organ blood flow measurements (Aukland and Wolgast, 1968). In the context of islet blood flow measurements hydrogen gas clearance possess the great advantage that it can measure blood flow in small volumes of tissue. This means that not only the whole islet organ can be studied, but also individual islets, since the size of our electrodes can be well accommodated into this small-sized structure. To demonstrate this, we manufactured platinum microelectrodes to measure hydrogen gas washout from individual endogenous and transplanted islets in the pancreas of male Lewis rats and in human and mouse islets implanted under the renal capsule of male athymic mice. Both in the rat endogenous pancreatic islets as well as in the intra-pancreatically transplanted islets, the vascular conductance and blood flow values displayed a highly heterogeneous distribution, varying by factors 6–10 within the same pancreas. The blood flow of human and mouse islet grafts transplanted in athymic mice was approximately 30% lower than that in the surrounding renal parenchyma. The present technique provides unique opportunities to study the islet vascular dysfunction seen after transplantation, but also allows for investigating the effects of genetic and environmental perturbations on islet blood flow at the single islet level in vivo.
islet blood perfusion in vivo, both in the endogenous pancreas and after transplantation. By this technique we could demonstrate a considerable heterogeneity in the blood flow values of both endogenous and intra-pancreatically implanted islets. We propose that this will become a valuable technique for evaluating the importance of islet blood flow changes in the pathogenesis of e.g. type 2 diabetes.

Material and methods

Animals

Adult male Lewis rats (250–300 g), C57BL/6 and C57BL/6 (nu/nu) mice (Tacoma, Ry, Denmark) with free access to tap water and pelleted food were used in the experiments. All experiments were approved by the Uppsala Committee on the Ethics of Animal Research (Permit Number: C108/11).

Islet isolation and culture

Rats were euthanized by exsanguination under anesthesia (intraperitoneal injection of pentobarbital; 60 mg kg⁻¹; Apoteksbolaget, Umeå, Sweden) and mice were euthanized by cervical dislocation under tribromoethanol anesthesia [Avertin, a 2.5% (vol/vol) solution of 10 g 97% 2,2,2-tribromoethanol (Sigma-Aldrich) in 10 ml 2-methyl-2-butanol (Kemila, Stockholm, Sweden)]. Pancreatic islets from rats and mice were isolated according to a previously described collagenase digestion method and hand-picked by the aid of a braking pipette (Johansson et al., 2006b). Groups of 150 islets were maintained free-floating in culture medium RPMI 1640 (Sigma-Aldrich, St. Louis, MO, USA) supplemented with l-glutamine (Sigma-Aldrich), benzylpenicillin (100 U ml⁻¹; Roche Diagnostics Scandinavia AB, Bromma, Sweden), streptomycin (0.1 mg/ml; Sigma-Aldrich) and 10% fetal calf serum (Sigma-Aldrich). The islets were cultured for 3–4 days, and the culture medium was changed every second day.

Human islets, isolated using standard methods (Ozmen et al., 2002; Ricordi et al., 1988), were obtained from the Department of Clinical Immunology, Uppsala, Sweden. All work involving human tissue was conducted according to the principles expressed in the Declaration of Helsinki and in the European Council’s Convention on Human Rights and Biomedicine. Consent for organ donation (for clinical transplantation) or the microsphere technique (non-transplanted rats).

Duct-ligation

The animals were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg; 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. A silk ligature was placed around these ducts, at the junction between the corpus and caput regions, and ligated (Jansson et al., 2005). The rats were then allowed to recover from anesthesia, and were kept single in cages for 7 days after which some of the animals were transplanted and then all were kept for another 4 weeks before being studied with hydrogen gas washout (transplanted rats) or the microsphere technique (non-transplanted rats).

Blood flow measurements with hydrogen gas clearance

Rats were anesthetized with an intraperitoneal injection of thiobutabarbitial (120 mg/kg; Inactin®; Sigma-Aldrich) and mice by tribromoethanol (Mattsson et al., 2002a) [Avertin, a 2.5% (vol/vol) solution of 10 g 97% 2,2,2-tribromoethanol (Sigma-Aldrich) in 10 ml 2-methyl-2-butanol (Kemila, Stockholm, Sweden)] and placed on a heated operating table. A tube was placed in the trachea and catheters were inserted into the left femoral artery to monitor mean arterial blood pressure (PDCR 75/1; Druck Ltd. UK) and the left jugular vein to infuse saline.

Hydrogen microelectrodes were custom-made platinum wire with an outer diameter of approximately 25 μm and were polarized at +0.18 V versus the reference electrode (Ag/AgCl), which was placed in abdominal muscle. The electrical current obtained is proportional to the hydrogen gas concentration (Aukland and Wolgast, 1968; Liss et al., 2005). The microelectrode was inserted by a micromanipulator under visual control into the renal islet grafts or into endogenous or transplanted islets. The duct-ligation enabled us to clearly visualize the islets under microscope and, therefore, ensured the intra-islet localization of the microelectrode. A small plastic tube was placed close to the tracheal tube and a flow of hydrogen gas was allowed to pass over the trachea. The flow was adjusted so that the mean arterial blood pressure was only marginally (10–15% decrease) affected.

After tissue hydrogen concentrations reached a saturation plateau (2–4 min), the supply of hydrogen was terminated, initiating clearance of the gas. The wash-out curve was recorded using hydrogen microelectrodes and was followed until it returned to control levels prior to gas loading (i.e. 2–5 min). In the subcapsular islet grafts, 2 measurements at different places in the implant were performed and the mean of these was considered to be one observation. In endogenous or transplanted single islets in the pancreas one measurement per islet was made. The number of measurements in each pancreas varied between 2 and 15 depending on the effects of hydrogen on blood pressure; a consistent decrease <15% caused us to terminate the experiments. At the end of the experiments mice were euthanized by cervical dislocation while rats by exsanguination via severing the arteria carotis.

The analysis of the wash-out curve was performed as previously described (Aukland and Wolgast, 1968; Liss et al., 2005). In short, the slope of the wash-out curve, consisting of 90% down to 50% of the maximal hydrogen current (100% equals the distance/values between hydrogen saturation down to control values, i.e. background), was analyzed for each wash-out curve recorded (Fig. 1A and 2A). Blood perfusion was calculated according to formula \( BF = \ln 2/TC \), where \( BF \) is blood flow, \( \ln 2 \) is the natural logarithm of 2 (0.693), and \( TC \) is the time for the \( H_2 \) current to decrease to 50%. The islet/graft vascular conductance, \( C \), was further calculated using the formula \( C = BF/BP \) where \( BP \) is the mean arterial blood pressure.
Blood flow measurements with microspheres

This technique has previously been described in detail (Carlsson et al., 2002). Briefly, the rats were anesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg kg$^{-1}$; Inactin®; Sigma-Aldrich). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5 °C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and the vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd.), thereby allowing constant monitoring of the mean arterial pressure. The animals breathed spontaneously through a tracheostomy.

This technique has previously been described in detail (Carlsson et al., 2002). Briefly, the rats were anesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg kg$^{-1}$; Inactin®; Sigma-Aldrich). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5 °C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and the vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd.), thereby allowing constant monitoring of the mean arterial pressure. The animals breathed spontaneously through a tracheostomy.

Blood flow measurements with microspheres

This technique has previously been described in detail (Carlsson et al., 2002). Briefly, the rats were anesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg kg$^{-1}$; Inactin®; Sigma-Aldrich). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5 °C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and the vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd.), thereby allowing constant monitoring of the mean arterial pressure. The animals breathed spontaneously through a tracheostomy.

Blood flow measurements with microspheres

This technique has previously been described in detail (Carlsson et al., 2002). Briefly, the rats were anesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg kg$^{-1}$; Inactin®; Sigma-Aldrich). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5 °C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and the vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd.), thereby allowing constant monitoring of the mean arterial pressure. The animals breathed spontaneously through a tracheostomy.

Blood flow measurements with microspheres

This technique has previously been described in detail (Carlsson et al., 2002). Briefly, the rats were anesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg kg$^{-1}$; Inactin®; Sigma-Aldrich). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5 °C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and the vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd.), thereby allowing constant monitoring of the mean arterial pressure. The animals breathed spontaneously through a tracheostomy.

Blood flow measurements with microspheres

This technique has previously been described in detail (Carlsson et al., 2002). Briefly, the rats were anesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg kg$^{-1}$; Inactin®; Sigma-Aldrich). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5 °C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and the vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd.), thereby allowing constant monitoring of the mean arterial pressure. The animals breathed spontaneously through a tracheostomy.

Blood flow measurements with microspheres

This technique has previously been described in detail (Carlsson et al., 2002). Briefly, the rats were anesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg kg$^{-1}$; Inactin®; Sigma-Aldrich). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5 °C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and the vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd.), thereby allowing constant monitoring of the mean arterial pressure. The animals breathed spontaneously through a tracheostomy.

Blood flow measurements with microspheres

This technique has previously been described in detail (Carlsson et al., 2002). Briefly, the rats were anesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg kg$^{-1}$; Inactin®; Sigma-Aldrich). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5 °C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and the vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd.), thereby allowing constant monitoring of the mean arterial pressure. The animals breathed spontaneously through a tracheostomy.

Blood flow measurements with microspheres

This technique has previously been described in detail (Carlsson et al., 2002). Briefly, the rats were anesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg kg$^{-1}$; Inactin®; Sigma-Aldrich). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5 °C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and the vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd.), thereby allowing constant monitoring of the mean arterial pressure. The animals breathed spontaneously through a tracheostomy.

Blood flow measurements with microspheres

This technique has previously been described in detail (Carlsson et al., 2002). Briefly, the rats were anesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg kg$^{-1}$; Inactin®; Sigma-Aldrich). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5 °C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and the vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd.), thereby allowing constant monitoring of the mean arterial pressure. The animals breathed spontaneously through a tracheostomy.

Blood flow measurements with microspheres

This technique has previously been described in detail (Carlsson et al., 2002). Briefly, the rats were anesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg kg$^{-1}$; Inactin®; Sigma-Aldrich). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5 °C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and the vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd.), thereby allowing constant monitoring of the mean arterial pressure. The animals breathed spontaneously through a tracheostomy.

Blood flow measurements with microspheres

This technique has previously been described in detail (Carlsson et al., 2002). Briefly, the rats were anesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg kg$^{-1}$; Inactin®; Sigma-Aldrich). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5 °C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and the vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd.), thereby allowing constant monitoring of the mean arterial pressure. The animals breathed spontaneously through a tracheostomy.

Blood flow measurements with microspheres

This technique has previously been described in detail (Carlsson et al., 2002). Briefly, the rats were anesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg kg$^{-1}$; Inactin®; Sigma-Aldrich). The animals were then placed on a heated operating table to maintain body temperature at approximately 37.5 °C. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery and the vein. The former catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd.), thereby allowing constant monitoring of the mean arterial pressure. The animals breathed spontaneously through a tracheostomy.
Endogenous and transplanted islets in the rat pancreas

Results

Statistical calculations

All values are given as means ± SEM. Probabilities of chance differences between the groups were calculated with Mann–Whitney’s rank-sum test or ANOVA with Bonferroni’s post-hoc test (SigmaPlot™; Systat Software, San Jose, CA, USA). The significant level of the tests is P < 0.05.

Results

Endogenous and transplanted islets in the rat pancreas

The duct-ligated rats tolerated the procedures well without any signs of infirmity. Blood glucose and serum insulin concentrations as well as hematocrit and mean arterial blood pressure were normal (Table 1). Atrophy of the exocrine tissue induced by duct-ligation made, as expected, identification of islets very simple, and we could easily identify endogenous from transplanted islets, with 20–30 islets of each kind observable. Thus, the platinum electrodes were easy to insert and we calculated both single islet blood flow and vascular conductance in these experiments. Administration of hydrogen led to a decrease (usually 10–15%) in mean arterial blood pressure. We therefore choose to express the values both as blood flow per se, and as vascular conductance.

The values for both endogenous and transplanted islets in the pancreas were quite heterogeneous and varied by factors 6–10 within the same pancreas (Figs. 1A and B). The heterogeneity was similar in all studied pancreases, as seen in Fig. 1B. The islet blood flow values were usually 2 ml/min × g islet tissue in both endogenous and transplanted islets, with occasional peak values of 4.5 ml/min × g islet (Fig. 1B). We divided the transplanted and endogenous islets into 4 subgroups depending on their vascular conductance, ranging from <0.01 ml/ups to >0.03 min × g/mm Hg (Fig. 1D). As can be seen more than 50% of the islets belonged to group with lowest conductance values and less than 10% to the group with highest conductance (Fig. 1D). Note that the transplanted islets were more numerous in these particular groups, while a larger fraction of endogenous islets had medium values for conductance (Fig. 1D). It should be noted that the above related differences in blood flow were not dependent on islet size, and that the studied islets, both transplanted and endogenous, had a diameter of 200–250 μm.

Endogenous islet volume (in percent) was 4–5 folds increased in the ligated part of the pancreas when compared to the non-ligated gland (Table 1). When microspheres were used in the duct-ligated and non-ligated part of the pancreas we measured the islet blood flow values in all islets in these parts of the pancreas simultaneously, and not in single islets. This is since it is difficult to apply the microsphere technique for single islet flow measurements as outlined further in the Discussion section. No differences in islet blood flow were seen when the duct-ligated and non-ligated parts of the gland were compared 5 weeks after ligation (Table 1). Islet blood flow, when measured by this technique and when expressed per whole islet organ, was 5–6 ml/min × g islets.

Table 1

<table>
<thead>
<tr>
<th>Duct or sham</th>
<th>Sham</th>
<th>Duct</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>340 ± 16</td>
<td>334 ± 18</td>
</tr>
<tr>
<td>After</td>
<td>382 ± 13</td>
<td>394 ± 16</td>
</tr>
<tr>
<td>Pancreas weight (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undiligated</td>
<td>422 ± 30</td>
<td>474 ± 55</td>
</tr>
<tr>
<td>Ligated</td>
<td>409 ± 24</td>
<td>404 ± 18</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>124 ± 6</td>
<td>120 ± 5</td>
</tr>
<tr>
<td>Blood glucose (mmol L⁻¹)</td>
<td>6.1 ± 0.2</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>Serum insulin (ng ml⁻¹)</td>
<td>2.19 ± 0.36</td>
<td>2.54 ± 0.48</td>
</tr>
<tr>
<td>Islet volume (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undiligated</td>
<td>0.82 ± 0.09</td>
<td>0.83 ± 0.19</td>
</tr>
<tr>
<td>Ligated</td>
<td>1.11 ± 0.10</td>
<td>5.19 ± 1.16</td>
</tr>
<tr>
<td>Islet mass (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undiligated</td>
<td>3.48 ± 0.49</td>
<td>4.06 ± 0.82</td>
</tr>
<tr>
<td>Ligated</td>
<td>4.54 ± 0.50</td>
<td>4.71 ± 0.55</td>
</tr>
<tr>
<td>Pancreatic blood flow (ml min⁻¹ g⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undiligated</td>
<td>10.95 ± 0.14</td>
<td>10.38 ± 0.17</td>
</tr>
<tr>
<td>Ligated</td>
<td>1.02 ± 0.10</td>
<td>3.97 ± 2.13</td>
</tr>
<tr>
<td>Islet blood flow (ml min⁻¹ mg⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undiligated</td>
<td>10.4 ± 1.8</td>
<td>8.2 ± 1.9</td>
</tr>
<tr>
<td>Ligated</td>
<td>9.5 ± 1.4</td>
<td>7.6 ± 1.6</td>
</tr>
<tr>
<td>Adrenal blood flow (ml min⁻¹ g⁻¹)</td>
<td>9.36 ± 0.64</td>
<td>8.02 ± 1.24</td>
</tr>
</tbody>
</table>

Values are means ± SEM.
Islet transplants under renal capsule

All islet grafts were easily visualized and identified and the electrodes could be inserted into the 2–3 mm large grafts. Administration of hydrogel led to a decrease (usually 10–15%) in mean arterial blood pressure also in mice. We therefore choose to express the values both as blood flow per se, and as vascular conductance. All these values were consistently lower in human islet grafts when compared to the adjacent renal parenchyma (Fig. 2). In some experiments we also evaluated syngeneic mouse islet grafts (n = 6), with similar findings (results not shown).

When studying separate animals (n = 4) with Laser-Doppler flowmetry, we could, as reported before (Mattsson et al., 2002b), verify that the measured tissue perfusion was approximately 30% lower in the human grafts than in the surrounding renal parenchyma (14.6 ± 2.0 vs. 21.7 ± 3.0 tissue perfusion units; P < 0.05). Blood glucose and serum insulin concentrations as well as hematocrit and mean arterial pressure were normal in these animals (results not shown).

Discussion

Pancreatic islet vasculature is important for normal islet function since it provides oxygen and nutrients for the metabolically active endocrine cells and disperses released hormones. Furthermore, islet endothelium constitutes a barrier between the blood stream and the endocrine niche (Otonkoski et al., 2008) and produces growth factors affecting beta-cell replication (Johansson et al., 2006a; Lammert et al., 2001). Previous studies have suggested that disturbed islet endocrine function is initially invariably associated with an increased islet blood perfusion (Jansson, 1994). Many factors co-operate to induce this hyperemia, and especially sympathetic nerves and released purines are important in this context (Jansson et al., 2010).

A major problem when examining islet blood flow is the lack of sensitive techniques for measuring single islet flow. The “gold-standard” of blood flow measurements, namely the microsphere technique, has a resolution which depends on the number of injected microspheres (Buckberg et al., 1971; Levine et al., 1984). Single islet blood flow is in the order of 20–50 nl min⁻¹, which is below the lower limit of microsphere detection in most instances but, despite this limitation, microspheres provide reliable results if the islet organ as a whole is studied (Jansson and Hellerström, 1984; Lifson, 1981; Lifson et al., 1980; Meyer et al., 1982). This also means that the distribution of microspheres follows a normal distribution, rather than a Poisson distribution (Buckberg et al., 1971; Hillerdal, 1987). The reason for this accuracy for whole islet organ measurements is naturally that the islet organ in e.g. rats constitutes several thousand of islets which when added together provides a volume constituting 1–2% of the whole pancreas which allows for the presence of a sufficient number of microspheres. Another disadvantage with the microsphere technique is that microsphere shunting may occur through islet capillaries, especially if they for some reason are dilated. This is dependent on the microsphere size, and provided that it exceeds 10 μm, this phenomenon is likely to be of less significance (Jansson and Hellerström, 1983; Jansson and Hellerström, 1984). However, recently hyperemic islets with markedly dilated capillaries have been described in human and porcine pancreas (Hilling et al., 2009). In islets grafted under the renal capsule, there is the additional problem of preferential streaming of microspheres (Morkrid et al., 1976), which overestimates the blood perfusion of subcapsularly transplanted islets, since a majority of the microspheres do not devastate into interlobular arteries, but remain in intralobular renal arteries. Thus, even if the microsphere technique is adequate for studies of whole organ islet blood flow, there are limitations to its use to measure single islet blood flow.

Semi-quantitative techniques that have been used to address islet blood flow measurements include in vivo microscopy (Menger et al., 2001; Nyman et al., 2008; Rooth et al., 1985), which allows mostly for studies of changes in blood perfusion rather than absolute measurements, and thus makes it difficult to compare values in different animals. Laser-Doppler flowmetry is difficult to use on single islets due to the size of the probes, but can be used to compare flow values in larger islet grafts (Sandberg et al., 1995). The disadvantage with this technique is the uncertainty of the volume of tissue penetrated by the laser light, and the risk of recording Doppler shifts also from erythrocytes in underlying tissues (Rajan et al., 2009).

Gas-washout techniques circumvent the problems referred to above. The major problem is instead detection of the gas within the tissues. We are able to prepare platinum microelectrodes with a size of 15–20 μm for the polarographic detection of hydrogen in tissues, which is perfectly adequate for insertion into an islet, the size of which is in the order of 200–250 μm. Islets of this size constitute the majority of the islet volume (Hellman, 1959), so we propose these to be representative also of the whole islet organ. One possible pitfall when placing the electrodes is their distance to larger blood vessels. If too close, the washout will be more rapid. However, normal islets do not contain any such large blood vessels (Bonner-Weir, 1993; Inn Veld and Marichal, 2010), and neither have we seen them after transplantation. Actually the blood flow values encountered in endogenous islets were remarkably similar among the pancreases from different animals and show that the technique can be reliably used. It should be noted, however, that for practical reasons we measured flow in a homogenous size group of islets, with a diameter of 200–250 μm. Interestingly, we found that blood flow values varied a lot among single islets transplanted within the same pancreas but also among the endogenous islets per se. Indeed, the basal blood perfusion varied 5–10 times between similarly sized islets in the same pancreas. These values are well in line with those showing also functional differences depending on the blood perfusion of the islets (Lau et al., 2012). The reasons for this are at present unknown, but may reflect differences in the regulatory properties of the islet vascular smooth muscle in different islets with implanted islets being largely denervated at this time after implantation (Korsgren et al., 1991). We have previously also reported that transplanted islets have a lower vascular density than endogenous islets (Mattsson et al., 2002b). The challenging topic to pursue next is if the blood perfusion of single islets correlates to the endocrine function and vascular reactivity of these islets. To address this issue we intend to use an ex vivo perfusion technique of single islets where we can directly study arteriolar vascular reactivity (Lai et al., 2007).

The studies on human islets grafted under the renal capsule of athymic nude mice, i.e. all islets are aggregated into a graft with a size of a few mm, confirmed our previous notion that the graft blood flow is lower than that of the surrounding implantation organ (Jansson and Carlsson, 2002). Interestingly the registered blood flow values/vascular conductance values were quite similar both within the same grafts and when grafts in different animals were compared, thereby confirming our previous findings.

Conclusions

The present study demonstrates that hydrogen washout technique is feasible for studies of blood perfusion of single islets as well as larger islet grafts. This will provide unique opportunities to study the islet vascular dysfunction seen after transplantation, but also will allow us to study effects of impaired glucose tolerance and overt diabetes on islet blood flow at the single islet level.

Conflict of interest

None declared.

Acknowledgments

This work was supported by the Swedish Heart and Lung Foundation (grant no. 20040645), an EFSD/Novo Nordisk (grant no. 300394), the Swedish Medical Research Council (grant no. 521-2011-3777).
References


