pH is decreased in transplanted rat pancreatic islets

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The recent introduction of a new treatment regimen, the so-called Edmonton Protocol, has markedly improved the outcome of clinical islet transplantation (3, 29). However, when this protocol is applied, transplantation of a large number of islets (>9,000 islet equivalents/kg body wt) is necessary to achieve insulin independence. In view of the limited availability of human islet tissue, different approaches to optimize the survival and function of the islet grafts are therefore clearly warranted to reduce the number of islets necessary to cure a diabetic individual.

Endogenous pancreatic islets have a complex glomerular-like angioarchitecture that ensures that no portion of an islet is more than one cell away from arterial blood (2). Furthermore, the blood perfusion of the pancreatic islets is markedly higher than that of the exocrine pancreas and approaches values similar to those of the renal cortex (5–7 ml/min⋅g−1) (4, 16). This unique capillary network and high blood perfusion secure a high delivery of oxygen and nutrients to the islet cells and optimize the dispersal of the secreted hormones to the vasculature. When islets are isolated and cultured before transplantation, the islet endothelium has been suggested to dedifferentiate or degenerate (25). In the immediate posttransplantation period, the islets are therefore supplied with oxygen and nutrients solely by diffusion from surrounding tissues (see Ref. 9).

The revascularization process is rapidly initiated, and the islets are revascularized within 7–14 days (20, 26). However, recent experiments on islets transplanted to kidney, liver, or spleen have suggested that this process is incomplete and that an oxygenation of the transplanted tissue similar to that in endogenous islets never occurs (6–8, 19). The metabolic consequences of this for the transplanted islets remain to be determined.

The aim of the present study, therefore, was to measure tissue pH in endogenous pancreatic islets and in islets transplanted under the renal capsule of diabetic and nondiabetic recipients before and after revascularization. We also recorded the oxygen tension (PO2) in these tissues and correlated this with the obtained pH values.

MATERIALS AND METHODS

Animals. The experiments were performed on inbred male Wistar-Furth rats weighing ~325 g and purchased from B&K Universal (Sollentuna, Sweden). The animals had free access to tap water and standard rat chow (R3, Ewos, Södermalje, Sweden) throughout the study. All experiments were approved by the animal ethics committee for Uppsala University.

Islet isolation, culture, and transplantation. Pancreatic islets were prepared by collagenase (Boehringer Mannheim, Mannheim, Germany) digestion, as described elsewhere (1). Groups of ~150 islets were cultured free-floating for 4–7 days in RPMI 1640 medium supplemented with 10% (vol/vol) calf serum (Sigma-Aldrich, St. Louis, MO) (1), and the medium was changed every 2nd day. At transplantation, ~250 islets were packed in a braking pipette and implanted beneath the renal capsule on the dorsal side of the left kidney in pentobarbital-anesthetized (60 mg/kg ip; Apoteket, Umeå, Sweden) syngeneic rats. Some of the recipients were treated with streptozotocin (STZ; 45 mg/kg iv; Sigma-Aldrich) 3–4 days before transplantation and were diabetic (blood glucose ~300 mg/dl) at the time of operation.
concentration >15 mmol/l) at transplantation. The number of transplanted islets was chosen to be insufficient to reverse the hyperglycemia in STZ-diabetic rats. Blood glucose concentrations were determined with test reagent strips (Medisense, Baxter Travenol, Deerfield, IL) from samples obtained from the cut tip of the tail.

Surgical procedures. The animals were anesthetized with an intraperitoneal injection of thiobutabarbital (120 mg/kg; Inactin, Research Biochemicals International, Natick, MA), placed on an operating table maintained at 37°C and tracheostomized. Polyethylene catheters were placed in the left femoral artery and vein. The arterial catheter was used to monitor blood pressure (Statlam P23dB, Statham Laboratories, Los Angeles, CA), whereas the catheter in the vein was used for infusion of Ringer solution (5 ml·kg⁻¹·h⁻¹) to compensate for loss of body fluid.

A left subcostal flank incision was made in the islet-transplanted animals. The left kidney was immobilized in a plastic cup attached to the operating table and embedded in pieces of cotton wool soaked in Ringer solution. The renal surface was covered with mineral oil (Kebo Grave) to prevent evaporation and keep the tissue moist and at body temperature. In control animals (not treated), an incision was made in the endogenous and transplanted islets with modified Clark-type microelectrodes (Unisense, Aarhus, Denmark) (6). The microelectrodes were polarized at −0.8 V, which gives a linear response between the PO₂ and the electrode current. The latter was measured by a picocamperimeter (University of Aarhus, Aarhus, Denmark). A two-point calibration of the electrodes was performed in air saturated with Na₂SO₃ or air at 37°C. The electrodes (tip OD diameter 10 μm) were inserted into the tissues with a micromanipulator under a stereomicroscope. The readings were allowed to stabilize for ~30 s. Stable recordings could then be obtained for long periods (>60 min). In the transplanted islets and surrounding renal cortex, ~10 measurements of PO₂ were performed in each animal. Measurements were performed at different depths from the tissue surface (250, 500, 750 μm). At least three measurements were performed at each depth. These locations were chosen on the basis of previous characterization of similar islet grafts (6). In the pancreas of control animals, measurements were performed in 3–5 superficial pancreatic islets and surrounding exocrine parenchyma. Multiple measurements were usually performed within the same islet; the mean was calculated to obtain the PO₂ value for one islet. The mean of all measurements in each tissue and animal was calculated and considered to be one experiment.

During the PO₂ measurements, blood pressure, body temperature, and tissue temperature (CT D85, Ellab, Copenhagen, Denmark) were continuously recorded with a MacLab instrument (AD Instruments, Hastings, UK) connected to a Power Macintosh 6100 computer. At the end of the experiments, arterial blood samples for analysis of hematocrit (Hct) and blood gases were obtained from the catheter in the femoral artery and collected in microhematocrit tubes (Kebo Grave).

**Graft insulin content.** The islet grafts were dissected free and placed in 1.0 ml of acid ethanol [0.18 M HCl in 95% (vol/vol) ethanol]. The grafts were sonicated to disrupt the islet cells, and the samples were then extracted overnight at 4°C, followed by determination of the insulin contents with an ELISA (rat insulin ELISA, Mercodia, Uppsala, Sweden).

**Lactate and pyruvate production in vitro.** Duplicate groups of 20 islets retrieved from 1-mo-old islet grafts (see previous paragraph) or freshly isolated control islets were incubated in sealed glass vials at 37°C in 250 μl of Krebs-Ringer bicarbonate buffer with HEPES containing 2 mg/ml albumin and 5.6 mmol/l n-glucose. The medium was refreshed with known pH 7.386 as air-5% CO₂ standardized culture conditions) or 1% O₂-94% N₂-5% CO₂ before incubation. The medium was removed after 2 h of incubation and stored at −20°C until analysis. Concentrations of lactate and pyruvate were measured with a microdialysis analyzer (CMA/600; CMA/Microdialysis, Stockholm, Sweden), which uses enzymatic reagents and colorimetric measurements at 546 nm. The incubation medium without islets was used as a blank.

**Statistical analysis.** All values are given as means ± SE. Multiple comparisons between data were performed by using ANOVA (Statview; Abacus Concepts, Berkeley, CA) and the Bonferroni post hoc test. Correlation analysis was obtained by simple linear regression. For all comparisons, P < 0.05 was considered to be statistically significant. Because the logarithmic pH scale does not allow parametric statistical evaluations, all recorded pH values were converted into hydrogen ion concentrations before the calculation of the means ± SEs. However, to facilitate interpretation of the data, these are presented as the corresponding pH values.

**RESULTS**

**Body weights and blood glucose concentrations.** All normoglycemic donors and recipients weighed ~325 g when the islet transplantations were performed (Table 1). Animals rendered diabetic by injection of STZ also weighed ~325 g, but they decreased ~10% in body weight until the time of islet transplantation 3–4 days later. After transplantation, normoglycemic animals slowly gained weight, whereas diabetic animals de-
creased in weight during the first posttransplantation week and thereafter leveled off at ~80% of their pretransplantation weight. All nondiabetic animals had a blood glucose concentration of 4–6 mmol/l. Administration of STZ made the animals hyperglycemic (blood glucose concentration >15 mmol/l) within 48 h. All STZ-treated animals remained diabetic when the tissue pH and PO2 measurements were performed.

**Graft insulin contents.** The islet grafts in the diabetic animals contained less insulin than the grafts in the nondiabetic recipients both 1 day and 1 mo after transplantation (Table 1). The insulin content in all islet grafts, i.e., grafts in both nondiabetic and diabetic recipients, decreased between 1 day and 1 mo after implantation.

**Arterial blood pressure, Hct, arterial PO2, and pH.** The mean arterial blood pressure was ~100 mmHg in all animals (Table 1). The Hct values ranged between 42 and 51% and did not differ between normo- and hyperglycemic animals. Likewise, neither arterial blood PO2 nor arterial blood pH differed between the groups.

**Tissue pH and PO2.** In the endogenous pancreatic islets, mean tissue pH was similar to that in arterial blood (Fig. 1). In the transplanted islets, tissue pH was markedly lower. Similar pH values were recorded in islet transplants in nondiabetic and diabetic animals and when investigated 1 day or 1 mo after transplantation. No differences in pH were found between different locations in the grafts (data not shown). Tissue pH in the exocrine pancreas and in the renal cortex adjacent to the implanted islet grafts (Fig. 2) was similar to the pH recordings in endogenous islets and arterial blood.

The mean PO2 in endogenous islets was ~35 mmHg (Fig. 3). Transplanted islets had a markedly lower tissue PO2 than endogenous islets when investigated both 1 day and 1 mo after transplantation. There was no statistically significant difference in PO2 between the islet grafts in nondiabetic and diabetic recipients at either time point. The decrease in tissue PO2 of the islet grafts in the nondiabetic recipients was aggravated between 1 day and 1 mo after transplantation. This difference, however, could not be seen in the diabetic recipients. No differences in PO2 were found between different locations in the grafts (data not shown). In the exocrine pancreatic parenchyma, mean tissue PO2 was 25–30 mmHg (Fig. 4). The PO2 in the renal cortex was ~20 mmHg in both nondiabetic and diabetic animals. There was a statistically significant negative correlation between the tissue PO2 and the hydrogen ion concentration in the 1-mo-old islet grafts in

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**Table 1.** Body weight, MAP, blood glucose concentration, graft insulin content, Hct, PO2, and pH of arterial blood when tissue oxygen tension and pH measurements are performed in control and islet-transplanted animals

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Nondiabetic</th>
<th>Diabetic</th>
<th>Nondiabetic</th>
<th>Diabetic</th>
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<tr>
<td>Body weight, g</td>
<td>325 ± 2</td>
<td>323 ± 2</td>
<td>291 ± 4‡</td>
<td>335 ± 6</td>
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<td>MAP, mmHg</td>
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<td>106 ± 3</td>
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<td>104 ± 5</td>
<td>108 ± 5</td>
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<td>Blood glucose, mmol/l</td>
<td>4.8 ± 0.2</td>
<td>4.6 ± 0.1</td>
<td>23.1 ± 0.5†</td>
<td>4.6 ± 0.2</td>
<td>22.4 ± 0.9‡</td>
</tr>
<tr>
<td>Insulin content, ng/graft</td>
<td>4.083 ± 0.57</td>
<td>1.298 ± 233†</td>
<td>2.324 ± 392‡</td>
<td>495 ± 101‡</td>
<td></td>
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<tr>
<td>Hct, %</td>
<td>46.0 ± 0.5</td>
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<td>P02, mmHg</td>
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<td>83 ± 3</td>
<td>81 ± 2</td>
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<tr>
<td>pH</td>
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<td>7.36 ± 0.01</td>
<td>7.36 ± 0.01</td>
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</tr>
</tbody>
</table>

Values are means ± SE for 6–8 experiments. Tx, transplanted; MAP, mean arterial blood pressure; Hct, hematocrit; PO2, oxygen tension; P02, arterial PO2; pH, pH of arterial blood. *P < 0.05 vs. control animals; †P < 0.05 vs. corresponding nondiabetic animals; ‡P < 0.05 vs. corresponding animals 1 day posttransplantation.

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Fig. 1. Tissue pH in endogenous and transplanted islets in nondiabetic (solid bars) and diabetic (hatched bars) Wistar-Furth rats. Measurements in transplanted islets were conducted 1 day or 1 mo after implantation of grafts. Note broken axis on ordinate. Values are means ± SE for 6–8 experiments. *P < 0.05 vs. endogenous islets. All comparisons were made using ANOVA.

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Fig. 2. Tissue pH in exocrine pancreas and in renal cortex adjacent to transplanted islets in nondiabetic (solid bars) and diabetic (hatched bars) Wistar-Furth rats. Note broken axis on ordinate. Measurements in transplanted animals were conducted 1 day or 1 mo after implantation of islet grafts. Values are means ± SE for 6–8 experiments.
diabetic animals (Fig. 5), but this could not be seen in the other experimental groups.

Lactate-to-pyruvate ratios. When exposed to standard culture conditions (95% air-5% CO₂), lactate-to-pyruvate (lactate/pyruvate) ratios in incubation medium from islets retrieved from islet grafts were increased compared with lactate/pyruvate ratios in the incubation medium of freshly isolated control islets (Fig. 6). The lactate/pyruvate ratios obtained from the incubation medium of retrieved islets exposed to 95% air-5% CO₂ were instead similar to those obtained from the incubation medium of freshly isolated control islets exposed to hypoxia (1% O₂). Islets retrieved from islet grafts and exposed to hypoxia had a markedly increased lactate/pyruvate ratio in the incubation medium compared with all other groups.

DISCUSSION

We have previously recorded a markedly decreased vascular density, blood flow, and tissue Po₂ in transplanted islets irrespective of whether implanted in the kidney, liver, or spleen (6–8, 19). In the present study, we measured tissue pH to assess the metabolic consequences of these changes. We found decreased pH and Po₂ in both 1-day-old and 1-mo-old islet transplants compared with endogenous pancreatic islets. Moreover, in diabetic recipients investigated 1 mo post-transplantation, islet graft hydrogen ion concentrations correlated negatively with tissue Po₂.

Accumulation of hydrogen ions is known to occur in tissues when a high glycolytic rate, and thereby lactic acid production, coincides with an insufficient drainage by convective and/or diffusive transport. Rapidly growing tumors often display severe tissue acidosis due to their high metabolic rate, which often cannot be adequately met by the nutrient blood supply (33). Po₂ values, as low as those recorded in the present and previous studies in transplanted islets, are in tumors often accompanied by a pH below 7.0 (12, 33). In contrast, tissue pH in the transplanted islets was not decreased by more than 0.11–0.15 pH units. However, whereas tumor cells have a high rate of lactate produc-
tion (e.g., see Refs. 24, 33), islet cells, and especially β-cells, have very low lactate dehydrogenase activity (17, 28). Nevertheless, in vitro experiments with islets incubated in a hypoxic environment have shown increased lactate production (18). In view of the very low lactate dehydrogenase activity in the β-cells, the produced lactate is likely to reflect mainly lactate production from non-β-cells in the grafts. In line with the present findings of a decreased pH in transplanted islets, we have in a recent microdialysis study noted 3–4 times higher lactate/pyruvate ratios in transplanted islets compared with islets investigated in vitro (5). As shown in the present study, these changes in metabolism observed in vivo seem to remain also immediately after retrieval of the transplanted islets. After incubation at standard culture conditions (95% air-5% CO₂), twofold higher lactate/pyruvate ratios were recorded in the medium of islets retrieved from islet grafts compared with freshly isolated control islets. Exposure of the retrieved islets to hypoxia (1% O₂) caused a further marked increase in lactate/pyruvate ratios. Although awaiting further study, this suggests changes in the enzymatic machinery. As evaluated in a previous study (30), pancreatic islets retrieved from islet grafts also seem to exhibit other functional disturbances, e.g., deteriorated glucose-stimulated insulin release.

Concomitant with the decreased tissue pH, a markedly decreased PO₂ in all transplanted islets, compared with endogenous islets, was found. In most cases, there was no strict correlation between the PO₂ and the pH in the tissues. The PO₂ in 1-mo-old islet transplants of normoglycemic recipients was lower than that in the corresponding 1-day-old islet transplants, whereas a similar difference was not observed in tissue pH. Nevertheless, in the 1-mo-old islet transplants in diabetic recipients, there was a negative correlation between the tissue PO₂ and hydrogen ion concentration. Interestingly, this experimental group also tended to have the lowest values for tissue PO₂ in the transplanted islets. It could be speculated that the rate of nonoxidative glucose metabolism is only proportional to the oxygen supply at this low range. Alternatively, the enzymatic machinery may be altered in transplanted islets when they are exposed to sustained hyperglycemia, as previously observed in remaining islets after an ~90% partial pancreatectomy (15). However, this awaits further study.

PO₂ values similar to those recorded in the islet transplants in the present study have previously been shown to affect islet function. In βTC3 cells, an insulinoma cell line, PO₂ values below 25 mmHg gradually shifted these cells from aerobic to anaerobic metabolism, with a concomitant increase in lactate production (23). Reduced insulin secretion from the βTC3 cells was observed at PO₂ < 7 mmHg. Similarly, glucose-stimulated insulin secretion from single rat islet cell aggregates (5–10 cells) was affected below a PO₂ of 12 mmHg (10). In vitro studies of pancreatic islets have also demonstrated a pivotal role of a near normal or slightly alkaline extracellular pH for glucose-stimulated insu-
REFERENCES


