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Angiotensin II type 1 receptor inhibition markedly improves the blood perfusion, oxygen tension and first phase of glucose-stimulated insulin secretion in revascularised syngeneic mouse islet grafts

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Abstract *Aims/hypothesis:* We recently found evidence of an angiotensin-generating system in pancreatic islets. The present study investigated the effect of endogenously produced angiotensin II on microcirculation and function in transplanted islets. *Materials and methods:* Losartan, an angiotensin II type 1 receptor inhibitor, was administered either acute intravenously to mice with 4-week-old islet renal subcapsular transplants, or added to the drinking water for the final 14 days or throughout the 4-week post-transplantation period. The graft-bearing kidney was, in some cases, dissected out and perfused in vitro to evaluate the effect of angiotensin II and losartan on glucose-stimulated insulin release from the grafts. *Results:* Losartan treatment throughout the 4-week post-transplantation period had negative effects on islet revascularisation as well as on islet graft insulin release. However, administration of losartan, either intravenously or orally, after the formation of a new vascular network, improved islet graft blood perfusion. P_{O2} in the islet transplants was also effectively improved by the losartan treatment. Graft perfusion experiments showed a markedly better first phase of glucose-stimulated insulin release in transplanted islets when exposed to losartan. In contrast, acute administration of angiotensin II decreased islet graft blood flow, PO_2 and glucose-stimulated insulin release. *Conclusions/interpretation:* This study shows that inhibition of the islet reninangiotensin system may be a feasible strategy to increase the blood perfusion, PO_2 and function within islet grafts. Such treatment should not be initiated, however, before the islet vascular system has been formed.

Keywords Angiotensin II · Islet transplantation · Losartan · Reninangiotensin system

Abbreviations AT_1: angiotensin II type 1 · RAS: reninangiotensin system

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Introduction

Modifications of islet allotransplantation protocols, as well as repeated transplantations to increase the implanted endocrine mass, have substantially increased the number of patients given the opportunity to become insulin independent [1, 2]. However, this concept further accentuates the already troublesome lack of human donors for clinical islet transplantation, and emphasises the need for optimising islet isolation techniques and treatments to ensure maximal survival and function of the grafted islets following transplantation. A potential contributing factor to poor graft function is insufficient or inadequate engraftment of the islets in the implantation organ. Normally, pancreatic islets have a rich blood supply that is almost ten times higher than that in the exocrine pancreas, and similar to that seen in the renal cortex (approx. 5–7 $ml \cdot min^{-1} \cdot g^{-1}$) [3]. However, isolation of pancreatic islets for transplantation dis-

rupts the islet vascular connections. Revascularisation of the transplanted islets is initiated within a few days and concluded within 14 days [4–6]. Nevertheless, blood perfusion and partial pressure of oxygen are chronically decreased in transplanted islets irrespective of implantation organ [7]. A reasonable explanation for the insufficient blood supply was provided in experiments where vascular density was found to be decreased in transplanted islets [8].

However, the vascular system of endogenous islets is not only characterised by a dense glomerular-like angioarchitecture but also a complex regulation of islet blood flow that closely adapts to fit the requirements for hormone secretion [3]. The functional properties of islet graft microvessels have not been described in as much detail. However, our previous studies have indicated that islet grafts may be even more sensitive than native islets to the vasoconstrictive effects of angiotensin II [9, 10]. Moreover, the presence of a local reninangiotensin system (RAS) in pancreatic islets [11] means that angiotensin II may be formed within islets in high local concentrations. The present study aimed to investigate the effect of endogenously produced angiotensin II on islet microcirculation and function in transplanted islets. For this purpose, losartan, an angiotensin II type 1 (AT_1) receptor inhibitor, was administered in one of the following ways: (1) acute intravenously to anaesthetised mice with renal subcapsular islet transplants; (2) added to the drinking water for the final 14 days, i.e. after the formation of a new islet vascular network; or (3) added to the drinking water throughout the 4-week post-transplantation period prior to the study.

Materials and methods

Animals

Male C57BL/6 mice weighing approximately 30 g were purchased from M&B (Ry, Denmark). The animals had free access to tap water and pelleted food throughout the study. All experiments were approved by the animal ethics committee of Uppsala University and followed the 'Principles of laboratory animal care' (NIH publication no. 85-23, revised 1985).

Islet isolation, culture and transplantation

Pancreatic islets from C57BL/6 mice were prepared by collagenase digestion and cultured free-floating for 4–5 days in groups of 150 islets in 5 ml RPMI-1640 medium supplemented with 11 mmol/l glucose and 10% (v/v) fetal calf serum in 95% air/5% CO_2 at 37°C [12]. The medium was changed every second day. At transplantation, approximately 200 islets were packed in a braking pipette and implanted beneath the renal capsule of the left kidney in avertin-anaesthetised mice (0.02 ml/g i.p. of a 2.5% [v/v] solution of 10 g 97% 2,2,2-tribromoethanol [Sigma, St Louis, MO, USA] in 10 ml 2-methyl-2-butanol [Kemila, Stockholm, Sweden]).

Chronic losartan treatment

Some of the transplanted mice were randomly assigned to treatment with losartan (30 $mg \cdot kg^{-1} \cdot day^{-1}$; Merck & Co, Whitehouse Station, NJ, USA) dissolved in the drinking water. The concentration of losartan in drinking water (0.13 mg/ml) to yield this dose of losartan was determined by pilot experiments (n=3) where the daily intake of water averaged 7 ml for each mouse during a 1-week period.

Oxygen tension and blood flow measurements

Four weeks after transplantation, some of the transplanted animals and some control (non-transplanted) animals were anaesthetised with avertin (0.02 ml/g i.p.), placed on an operating table maintained at 37°C, and surgically prepared for islet oxygen tension and blood flow measurements, as previously described [13, 14].

Oxygen tension was measured in the endogenous and transplanted islets as well as in the surrounding tissues using modified Clark-type microelectrodes [14]. The electrodes (tip outer diameter 2–6 μm) were inserted into the tissues using a micromanipulator. At least ten measurements of PO_2 were taken from the transplanted islets and surrounding renal parenchyma in each animal. In the pancreata of control animals, measurements were performed in three to five superficial pancreatic islets and the surrounding exocrine parenchyma. Multiple measurements were usually performed within the same islet; the mean was calculated to obtain the PO_2 value for one islet. The mean of all measurements in each tissue and animal was calculated and considered to be one experiment. Measurements of oxygen tension in transplanted islets were in some cases performed before and 10 min after an intravenous injection of losartan (10 mg/kg body weight; Merck & Co.), or before and after a 10-min infusion of angiotensin II (10 ng/min; Sigma).

In conjunction with the oxygen tension measurements, the blood perfusion of the islet graft and the adjacent renal cortex was measured by laser-Doppler flowmetry (PF4001-2; Perimed, Stockholm, Sweden) using a needle probe (411; tip outside diameter 0.45 mm; Perimed). The flow probe was positioned perpendicular to the immobilised tissue surface using a micromanipulator, and care was taken not to cause any compression of the tissue. At least four blood flow measurements were performed in the transplanted islets and renal cortex in each animal both before and after administration of losartan or angiotensin II (see above section). The mean of these measurements from each animal was calculated and considered to be one experiment. All blood flow values are given as arbitrary tissue perfusion units, as it is difficult to calibrate the instrument in physical units of blood flow. The blood perfusion of endogenous islets cannot be determined by laser-Doppler

flowmetry because of their small size. During the oxygen tension and blood flow measurements, blood pressure, body temperature and tissue temperature were continuously recorded using a MacLab Instrument (AD Instruments, Hastings, UK). All measurements of oxygen tension and blood perfusion were, for obvious technical reasons, only performed at one time point in each experimental animal.

Measurements of blood parameters

Blood glucose concentrations were determined using test reagent strips (Medisense, Baxter Travenol, Deerfield, IL, USA) from samples obtained from the cut tip of the tail. At the end of the blood flow and oxygen tension measurements, a blood sample was collected for analysis of haematocrit and blood gases. Haematocrit lower than 40, pH lower than 7.30, PO_2 lower than 10 kPa, or PCO_2 higher than 6.8 kPa were used as exclusion criteria, which led to the exclusion of one animal from the study owing to CO_2 retention.

Light microscopic evaluation

The graft-bearing left kidneys, or pancreata from control animals, were removed after the oxygen tension and blood flow measurements, fixed in 10% (v/v) formaldehyde and embedded in paraffin. Sections (5 μm thick) of the islet grafts and pancreata were prepared and stained with the lectin *Bandeiraea simplicifolia* (Sigma) [8]. The slides were counterstained with haematoxylin. For each animal, ten or more tissue sections from all parts of the pancreas, or islet transplants stained with *Bandeiraea simplicifolia*, were randomly chosen and evaluated. The number of stained blood vessels in endogenous and transplanted islets was quantified using a light microscope (magnification ×100). In the islet grafts, connective tissue surrounded the individual islets in the grafts. The blood vessels in the endocrine and connective tissues were therefore counted separately. The fraction of the islet grafts composed of endocrine tissue was measured by a direct point-counting method. The intersections overlapping connective tissue stroma and endocrine cells within the islet grafts were counted at a magnification of ×100 using a light microscope. The area of the investigated endogenous and grafted islets was determined using a computerised system for morphometry (Easy image 3000; Tekno Optik, Huddinge, Sweden). Vascular density, i.e. the number of stained blood vessels found per measured islet or graft area (mm^2), was then calculated.

Islet graft perfusion

Grafts from some of the transplanted animals were investigated 4 weeks after transplantation for glucose-stimulated insulin secretion using a previously published protocol [15]. Briefly, the graft-bearing left kidney was removed together with part of the aorta and inferior vena cava. The ureter and the renal vein were cut, while the aorta was cannulated and infused with a continuously gassed (O_2:CO_2=95:5) Krebs–Ringer bicarbonate buffer supplemented with 2.0% (w/v) of each of BSA (fraction V; Miles Laboratories, Slough, UK) and dextran T70 (Pharmacia, Uppsala, Sweden) as well as with 2.8 or 16.7 mmol/l glucose respectively. The medium was administered at a rate of 1.0 ml/min without recycling for 60 min with a perfusion pressure of approximately 40 mmHg. The perfusion experiments started with a 15-min period using a medium containing 2.8 mmol/l glucose, followed by 30 min using 16.7 mmol/l glucose and 15 min using 2.8 mmol/l glucose. In some experiments, angiotensin II (Sigma) or losartan (Merck) was added during the perfusion with the high-glucose medium (16.7 mmol/l) at a concentration of 10 ng/ml or 100 μg/ml respectively. A 1.0-ml sample of the effluent medium was collected every fifth minute, except during the first 10 min of perfusion with the high glucose concentration, when samples were taken after 1, 2, 3, 4, 5, 7 and 10 min. The insulin concentrations of the effluent samples were measured by ELISA (Mercodia, Uppsala, Sweden). The rate of insulin secretion was calculated by multiplying the insulin concentration in the sample by the flow rate, giving values of insulin expressed as ng/min.

Insulin contents of the islet grafts

After perfusion the graft was removed from the kidney and homogenised in 1,000 μl of acid-ethanol (0.18 mmol/l HCl in 95% [v/v] ethanol). The samples were extracted overnight at 4°C, followed by ELISA of the insulin (Mercodia).

Islet graft (pro)insulin and total protein biosynthesis

Four weeks after transplantation, some of the transplanted animals were allocated for measurement of islet graft (pro) insulin and total protein biosynthesis, as previously described [15]. Briefly, explanted islet grafts were incubated with or without 1 μmol/l losartan (Merck) at 37°C in 100 μl of Krebs–Ringer bicarbonate HEPES buffer containing 1.85 MBq/ml of L-[4,5,$^{-3}$H]leucine (Amersham-Pharmacia Biotech, Uppsala, Sweden) and 16.7 mmol/l glucose in an atmosphere of humidified air plus 5% CO_2. After 2 h, the islets were washed in buffer containing non-radioactive leucine (10 mmol/l) and sonicated in 200 μl of redistilled water. The amount of labelled (pro)insulin was determined by an immunoadsorption technique [16] and the total protein biosynthesis was measured in trichloroacetic acid precipitates of islet homogenates.

Statistical analysis

All values are given as means±SEM. When multiple comparisons between data were performed, one-way ANOVA and Bonferronni's post-hoc test were applied. For pairwise

comparisons, Student's *t*-test was used. For all comparisons, a *p* value of less than 0.05 was considered statistically significant.

Results

Mean arterial blood pressure and blood glucose concentrations

Data are shown in Table 1. All non-pretreated animals allocated to studies of blood flow and oxygen tension had a mean arterial pressure of 100–110 mmHg. Acute intravenous administration of angiotensin II increased blood pressure by approximately 25%, while acute intravenous or daily oral losartan treatment decreased the blood pressure to between 80 and 90 mmHg. Blood glucose concentrations were similar in all animals and were not affected by angiotensin II or losartan treatment.

Blood flow in islet grafts and the renal cortex

Data are shown in Fig. 1. Before treatment, the blood perfusion of the islet grafts was approximately 33% of that of the renal cortex in all animals. Acute intravenous administration of angiotensin II decreased the blood perfusion by approximately 30% in the islet grafts and the renal cortex. Acute intravenous losartan administration increased islet graft blood flow by 175±37% (n=6) but renal cortical blood flow by only 75±10% (n=6) (p<0.05). Thus, after acute losartan treatment, islet graft blood flow was approximately 50% of that in the renal cortex. Losartan added to the drinking water throughout the 4-week post-transplantation period did not affect islet graft blood flow or renal cortical blood flow. In contrast, when administered orally during the final 14 days, i.e. after the formation of a new vascular network, islet graft and renal blood perfusion were increased to a similar extent to that seen following acute intravenous treatment.

Oxygen tension in islet grafts and the renal cortex

Data are shown in Fig. 2. Oxygen tension in the 4-week-old islet transplants was, in all cases, lower than 10 mmHg in

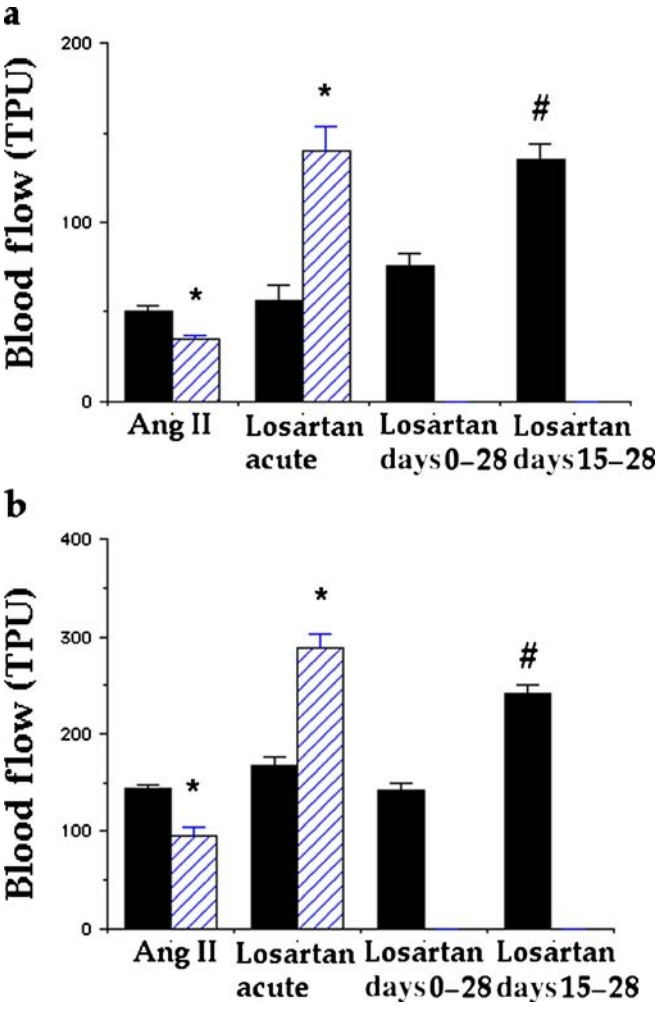


Fig. 1 Effect of intravenous infusion of angiotensin II (*Ang II*; 10 ng/min), intravenous injection of the angiotensin II type 1 receptor inhibitor losartan (10 mg/kg; acute) and oral treatment with losartan (30 mg·kg^{-1}·day^{-1}) dissolved in the drinking water throughout the post-transplantation period (*days 0–28*) or for the final 14 days (*days 15–28*) on blood flow in islet graft (**a**) and adjacent renal cortex (**b**) in C57Bl/6 mice 4 weeks after transplantation. *Closed* and *hatched bars* show results before and after intravenous injections respectively. Please note the different scales on the ordinates in (**a**) and (**b**). All values are means±SEM for six to eight animals. *p<0.05 vs before treatment; $^{\#}p$<0.05 vs non-pretreated transplanted animals. Values from the groups 'before acute angiotensin II' and 'before acute losartan treatment' were pooled when used as control for the orally treated losartan groups. All comparisons were made using ANOVA. *TPU* Tissue perfusion units

Table 1 Mean arterial blood pressure (MAP) and blood glucose concentrations in non-transplanted and islet-transplanted C57BL/6 mice allocated to studies of islet blood flow and oxygen tension

	Non-transplanted animals	Transplanted animals					
		Angiotensin II		Losartan		Losartan pretreatment	
		Before	After	Before	After	Days 0–28	Days 15–28
MAP (mmHg)	99±7	98±3	124±4[a]	112±3	89±2[a]	88±3[a]	82±2[a]
Blood glucose (mmol/l)	7.2±0.1	7.2±0.2	7.0±0.4	6.9±0.1	6.7±0.1	7.3±0.4	7.2±0.3

All values are the means±SEM for six to eight animals in each group
All comparisons were made using ANOVA and Bonferroni's posthoc test
[a]p<0.05 vs the corresponding untreated animals

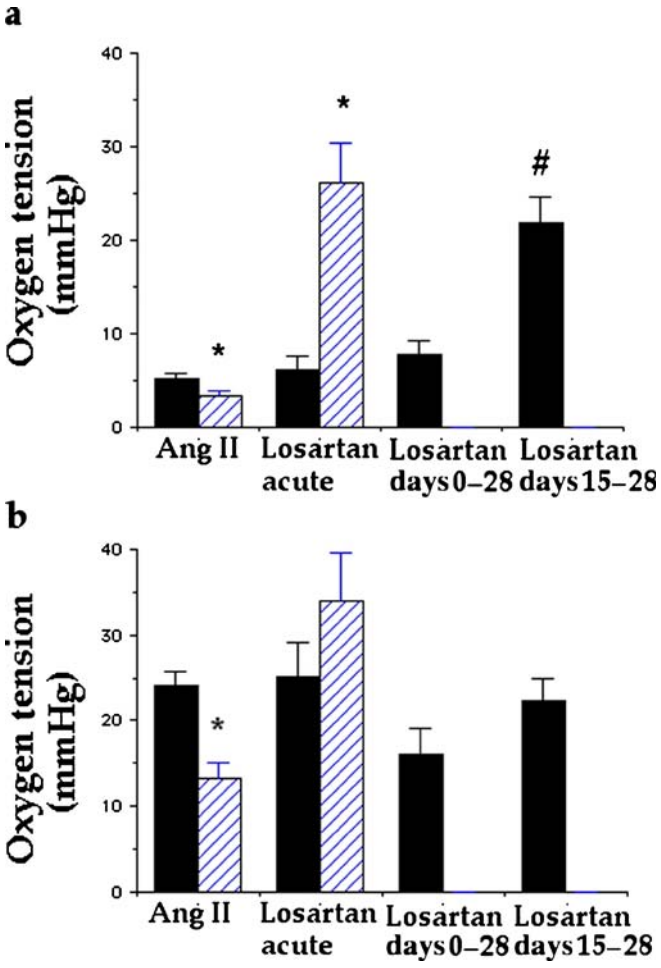


Fig. 2 Effect of intravenous infusion of angiotensin II (*Ang II*; 10 ng/min), intravenous injection of the angiotensin II type 1 receptor inhibitor losartan (10 mg/kg; acute) and oral treatment with losartan (30 mg kg^{-1} day^{-1}) dissolved in the drinking water throughout the post-transplantation period (*days 0–28*) or for the final 14 days (*days 15–28*) on oxygen tension in islet graft (**a**) and adjacent renal cortex (**b**) in C57BL/6 mice 4 weeks after transplantation. *Closed* and *hatched bars* show results before and after intravenous injections respectively. All values are means±SEM for six to eight animals. *$p<0.05$ vs before treatment; $^{\#}p<0.05$ vs non-pretreated transplanted animals. Values from the groups 'before acute angiotensin II' and 'before acute losartan treatment' were pooled when used as control for the orally treated losartan groups. All comparisons were made using ANOVA

untreated animals (Fig. 2a) and therefore in less than 20% of values in native islets (44.3±2.5 mmHg; n=6). Acute intravenous administration of angiotensin II decreased the oxygen tension levels in both the islet grafts and the adjacent renal cortex. Acute intravenous losartan treatment markedly improved the P_{O2} in the islet transplants. Oxygen tension in the renal cortex also tended to be slightly increased after acute losartan treatment, but this increase was not statistically significant (p=0.27). Chronic losartan treatment throughout the 4-week period following transplantation had no effect on the oxygen tension in the islet grafts or the renal cortex. However, when losartan was administered orally during the final 14 days prior to measurements, the P_{O2} in the islet transplants was improved while renal cortical P_{O2} was unaffected.

Vascular density

Data are shown in Figs 3 and 4. The vascular density in the transplanted islets of untreated animals, and of animals treated orally with losartan for the final 14 days, was 20–25% of that in native islets. Individual islets in the grafts, however, were surrounded by connective tissue stroma with a high number of capillaries. The total islet graft vascular density, i.e. the combined vascular density of the connective and endocrine compartments of the grafts, was therefore similar to that in native islets. Losartan treatment throughout the post-transplantation period, also including the revascularisation phase, selectively decreased the vascular density in the endocrine parts of the grafts. Oral losartan treatment also decreased the connective tissue percentage in the islet grafts from 36.9±3.2% (n=8) to 21.6±3.3% (n=7; treatment throughout the post-transplantation period) and 13.5±1.4% (n=7; treatment for the final 14 days) ($p<0.05$ for both comparisons vs control). As there was less connective tissue in the islet grafts obtained from animals treated with losartan for the final 14 days before study, the total islet graft vascular density in these grafts was lower than in control grafts.

Graft insulin release and insulin contents

Data on graft insulin release are shown in Fig. 5. A first-phase insulin release in response to a high glucose stim-

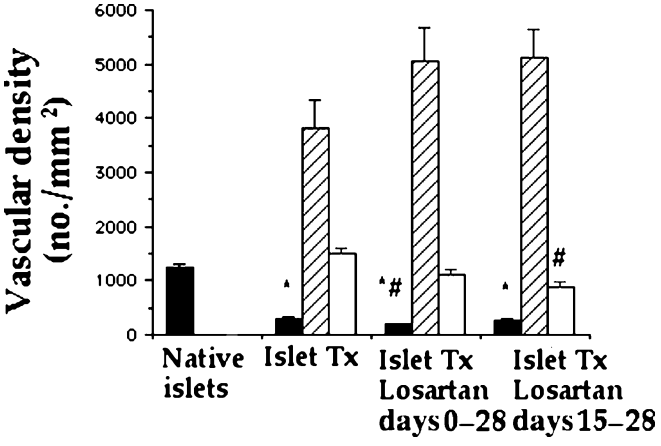


Fig. 3 Vascular density in native mouse pancreatic islets and in islet grafts 4 weeks after transplantation beneath the renal capsule of syngeneic C57BL/6 mice. Some of the transplanted animals were subjected to treatment with the angiotensin II type 1 receptor inhibitor losartan (30 $mg \cdot kg^{-1} \cdot day^{-1}$) dissolved in the drinking water throughout the 4-week post-transplantation period (*days 0–28*) or for the final 14 days (*days 15–28*). Values are given for the endocrine tissue (*closed bars*), the connective tissue stroma (*hatched bars*) and the whole islet graft (*empty bars*). All values are means±SEM for six to eight animals. *$p<0.05$ vs native pancreatic islets; $^{\#}p<0.05$ vs endocrine tissue in control islet grafts. All comparisons were made using ANOVA. *Tx* Transplant

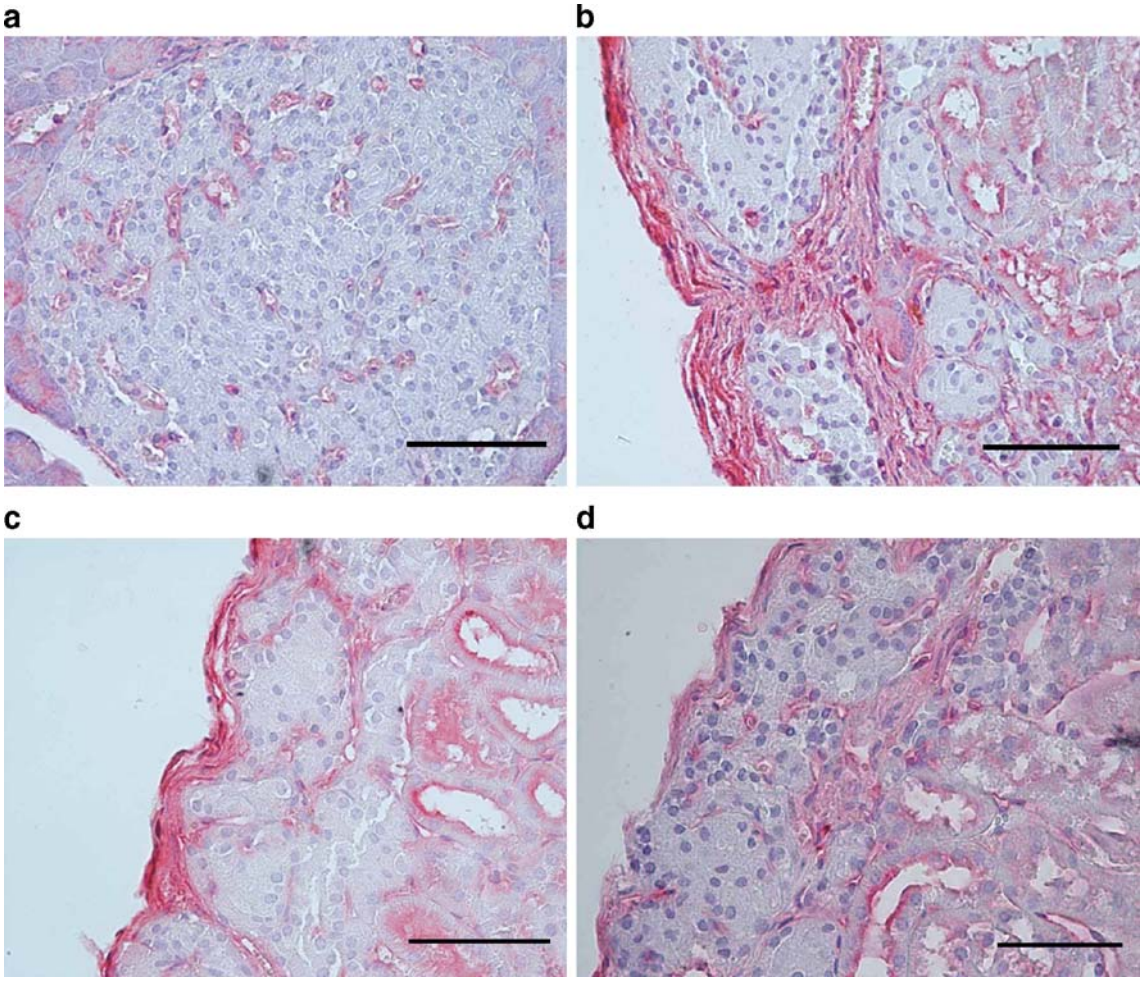


Fig. 4 Micrographs of native pancreatic islet (**a**) and 4-week-old islet grafts retrieved from untreated animals (**b**) or from animals treated with losartan throughout the post-transplantation period (**c**) or for the final 14 days (**d**). All tissues were stained with the lectin *Bandeirae simplicifolia* (*red*) to visualise blood vessels within the pancreatic islets. Scale bar=50 μm

ulation (15–20 min) and a sustained second phase (21–45 min) were observed in otherwise untreated islet grafts. Adding angiotensin II to the high-glucose medium during perfusion attenuated the first phase of glucose-stimulated insulin release, and also decreased insulin release early in the second phase (Fig. 5a). When losartan was added to the high-glucose medium, there was a markedly improved early phase of insulin release with a peak value more than double that in control grafts (Fig. 5b). In contrast, the second phase of insulin release was decreased in these acute losartan-treated grafts. Islet grafts obtained from transplanted animals subjected to losartan treatment throughout the 4-week post-transplantation period had a lower first and second phase of insulin release than control grafts. However, when losartan was administered orally during the final 14 days prior to measurements, the first phase of glucose-stimulated insulin release was increased while the second phase was decreased.

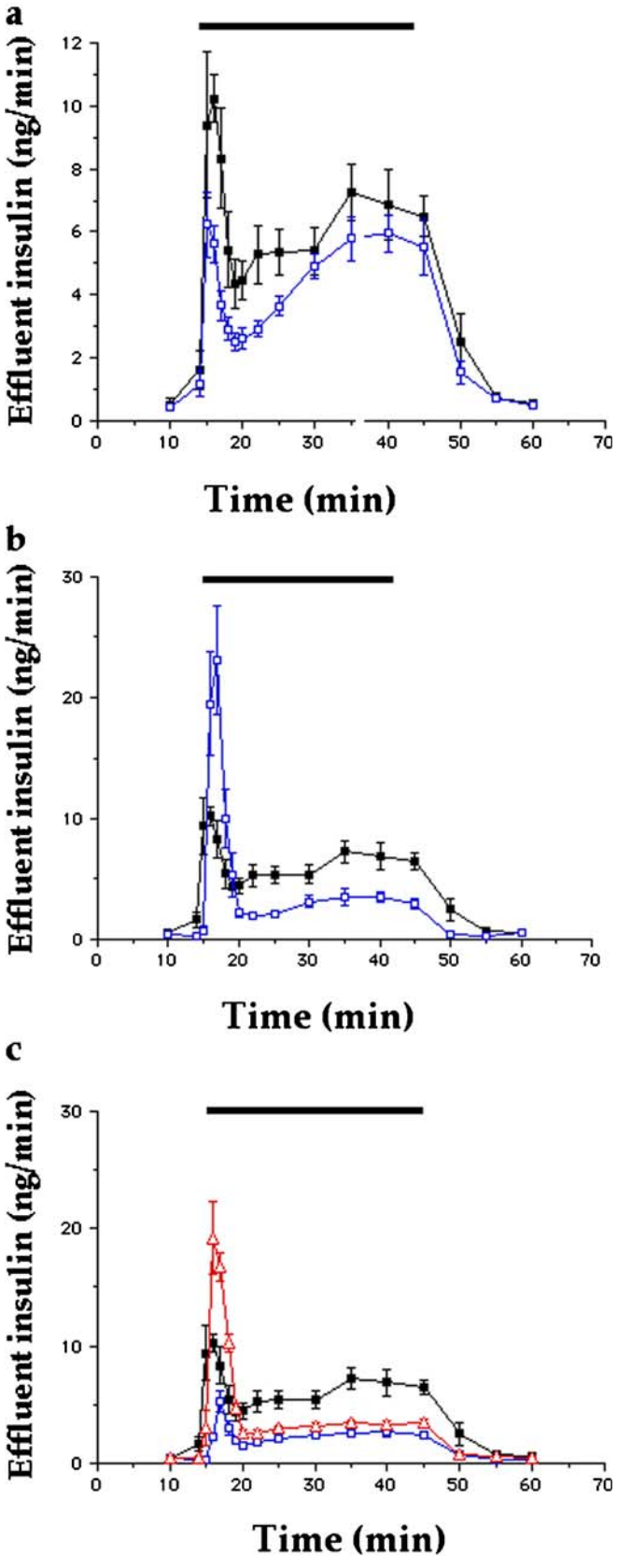
The insulin content in control islet grafts was 3,105±202 ng insulin/graft (n=8). Islet grafts in animals subjected to oral losartan treatment throughout the 4-week post-transplantation period or during the final 14 days before measurements had higher insulin contents (4,568±211 [n=8] and 5,051±204 ng insulin [n=8] respectively, $p<0.05$ in both cases) when compared with controls.

Graft (pro)insulin and total protein biosynthesis

Exposure to 1 μmol/l losartan did not affect (pro)insulin biosynthesis in retrieved islet grafts at high glucose concentrations (16.7 mmol/l; 512±83 [n=7] and 550±95 [n=8] dpm·islet graft^{-1}·120 min^{-1} for control and losartan-exposed grafts respectively). Likewise, total protein biosynthesis in the islet grafts was not affected by losartan at the high glucose concentrations (803±77 and 936±231 dpm·islet graft^{-1}·120 min^{-1} for control and losartan-exposed grafts respectively; n=7 in both groups).

Discussion

Several organs including the pancreas seem to have local RAS, where locally produced angiotensin II exerts local



◀ **Fig. 5** Insulin concentrations in effluent medium collected from perfused islet-graft-bearing kidneys of C57BL/6 mice. After 15 min of perfusion with a medium containing 2.8 mmol/l D-glucose, insulin secretion was stimulated by a 30-min period with 16.7 mmol/l D-glucose (*bar*) followed by 15 min with 2.8 mmol/l D-glucose. In (**a**) the effect of adding angiotensin II (10 ng/ml; *empty boxes*) to the high-glucose medium was compared with the perfusion of control islet grafts (*closed boxes*). $p<0.05$ vs control grafts for all observations between 15 and 25 min. In (**b**) the effect of adding the angiotensin II type 1 receptor inhibitor losartan (100 μg/ml; *empty boxes*) to the high-glucose medium was compared with the perfusion of control islet grafts (*closed boxes*). $p<0.05$ vs control grafts for all observations between 16 and 18 min and between 20 and 45 min. In (**c**) the effect of oral treatment with losartan throughout the post-transplantation period (days 0–28; *empty boxes*) or for the final 14 days (days 15–28; *empty triangles*) was compared with the perfusion of control islet grafts (*closed boxes*). $p<0.05$ vs control grafts for losartan days 0–28 between 16 and 50 min, for losartan days 15–28 between 16 and 18 min, and for losartan days 15–28 between 20 and 45 min. Please note that the same controls are used in **a**–**c**, but that the scales on the ordinates are different. All values are given as means±SEM for six to eight animals. $p<0.05$ vs control grafts. All comparisons were made using ANOVA

effects [17–24]. The best known functions of angiotensin II, e.g. cardiovascular effects, are mediated through the AT_1 receptor [25]. The clinical use of RAS inhibitors in the treatment of hypertension and cardiac failure shows few adverse effects. On the contrary, mainly beneficial effects, such as reduced cardiovascular morbidity and mortality and reduced diabetes incidence, have been described [26]. Mouse pancreatic islets were recently shown to have a local RAS, which means that angiotensin II may be formed locally in high concentrations within the islets [11]. Moreover, the AT_1 receptor for angiotensin II was markedly upregulated in islet transplants [11].

In previous studies of native islets, we observed that exogenous administration of angiotensin II may inhibit first-phase glucose-stimulated insulin release through vasoconstrictive effects [9]. The vasoconstrictive effects of angiotensin II in transplanted islets seemed even more pronounced than those in native islets, as previously observed [10]. In the present study, we found that the oxygenation and the first and second phases of glucose-stimulated insulin release were decreased by angiotensin II in transplanted islets. Some of the effects of exogenously administered angiotensin II, especially on the second phase, may not just be related to vascular effects but may be direct negative effects on (pro)insulin biosynthesis, as recently reported [11].

Administration of an angiotensin-converting enzyme inhibitor or an angiotensin II receptor antagonist increases native islet blood flow two- to three-fold [9]. The present findings show that islet graft blood flow may be improved to a similar extent after blocking the AT_1 receptor for angiotensin II. Most interestingly, the increase in islet graft blood perfusion improved the oxygen tension levels within the transplanted pancreatic islets and also augmented the first phase of glucose-stimulated insulin release. Thus, in contrast to in native islets [9], inhibiting the actions of endogenously formed angiotensin II in transplanted islets not only causes increased blood perfusion but also marked improvements in early insulin release. Such functional

improvement has also recently been described in islets of the type 2 diabetic Zucker rat [27]. The improved first phase of glucose-stimulated insulin release in our experiments may be caused by direct effects of losartan on pancreatic beta cells, but this seems less likely in view of the fact that losartan, in our previous experiments in this strain [11], had no acute effects on glucose-stimulated insulin release, glucose oxidation or (pro)insulin biosynthesis in isolated islets. The latter finding was also confirmed for retrieved islet grafts in the present study. This raises the possibility that the low blood perfusion of transplanted islets normally restricts and delays the output of insulin to the systemic circulation. The lower insulin release in the second phase of losartan-exposed islet transplants would then be explained by a 'post-washout' effect. The improved first phase of glucose-stimulated insulin exocytosis may also be the result of the higher oxygenation of the islet beta cells, secondary to the higher blood perfusion. The normally low oxygenation of transplanted islets means that their metabolism tends to be much more anaerobic than that of endogenous islets, which results in lactate formation and tissue acidosis [28]. It is interesting to note that the kinetics of glucose-stimulated insulin secretion from the losartan-exposed islet grafts, with its distinct first peak, mimics the perfused mouse pancreas much more than control islet grafts [29, 30].

Notably, not only islet graft blood flow but also renal blood flow was increased by losartan in this study. Although the increase in renal blood flow was less pronounced, it suggests that the observed increase in islet graft blood flow is site dependent. Previous studies, however, strongly indicate that the blood flow regulation of islet grafts is independent of the implantation organ [31, 32].

Besides the well-known effects on vascular tonus, several studies implicate a role of local RAS in oxidative stress defence [33, 34] and in the regulation of angiogenesis [35–38]. To evaluate the effect of islet RAS on the formation of a new islet vascular system after transplantation, losartan was, in some cases, added to the drinking water of newly transplanted animals. Losartan notably hindered the islet revascularisation process. The inhibitory effects of losartan on angiogenesis in the islet grafts seemed specific to the endocrine tissue. The difference between the endocrine and connective tissue is unlikely to be explained by the effect of losartan on angiogenesis, but may be a result of inhibiting the effects of locally formed islet angiotensin II. Notably, the grafts exposed to losartan throughout the 4-week post-transplantation period had decreased first and second phases of glucose-stimulated insulin release in perfusion experiments, despite a blood perfusion similar to that found in control grafts. This suggests that not only the level of islet graft blood flow but also the localisation of blood vessels in relation to the insulin-secreting beta cells is highly important for the insulin drainage. The insulin content in the pancreatic islets, chronically exposed to losartan, was higher than that in transplanted control islets, which may reflect improved oxidative stress defence with the survival of more islet cells. The lower proportion of connective tissue in islet grafts subjected to chronic losartan treatment most likely reflects inhibition of the pro-fibrotic properties of angiotensin II mediated through the AT_1 receptor [39].

The reason for the seemingly increased importance of a local angiotensin-generating system in transplanted islets compared with in endogenous islets is unclear. However, at least in the exocrine pancreas, inflammatory conditions upregulate several components of the local RAS [40]. Moreover, high local expression of angiotensin II may be maintained or even upregulated by chronically low levels of oxygen tension [33, 41], which seem to occur in islet grafts [42].

In conclusion, this study shows that inhibition of the local islet RAS may be a feasible strategy to increase blood perfusion, oxygen tension and function within islet grafts. Indeed, the impairments in the islet graft vascular system seem to be a combination of anatomical failure with low vascular density, and functional failure with inappropriately low blood perfusion.

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