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Evidence for a local angiotensin-generating system and dose-dependent inhibition of glucose-stimulated insulin release by angiotensin II in isolated pancreatic islets

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Abstract

Aims/hypothesis. A local angiotensin-generating system has been found in the exocrine pancreas. This study aimed, primarily, to investigate the existence of a local angiotensin-generating system in the pancreatic islets and, secondly, to elucidate its role in regulating insulin secretion.
Methods. Real-time RT-PCR and western blot were used to investigate if angiotensin-generating components are present in the mouse pancreatic islets, which are subject to regulation by islet transplantation. The localisation of AT_1-receptors in islets was investigated by immunohistochemistry. Batch-type incubations of isolated islets were applied for studying the influence of angiotensin II on the glucose-stimulated insulin release, glucose oxidation and (pro)insulin, and total protein biosynthesis.
Results. Major components, namely angiotensinogen, *ACE*, AT_1- and AT_2-receptors, were expressed in endogenous islets. AT_1-receptors were localised to pancreatic beta cells. Exposure of the isolated islets to angiotensin II induced a dose-dependent inhibition of glucose-stimulated insulin release and inhibited (pro)insulin biosynthesis. This inhibitory action was fully preventable by pretreatment of the islets with losartan, an AT_1-receptor antagonist. We also investigated if the expression of these components was changed after islet transplantation. Notably, a markedly increased expression of mRNA for the AT_1 -receptor was observed in islets retrieved from 4-week-old syngeneic islet transplants, a finding that was confirmed at the protein level.
Conclusion/interpretation. These data indicate the existence of an islet angiotensin-generating system of potential importance in the physiological regulation of glucose-induced insulin secretion, thus diabetes mellitus. The increased expression of the AT_1-receptor in islet transplants could have relevance to islet-graft function. [Diabetologia (2004) 47:240–248]

Keywords Losartan · Insulin biosynthesis · AT_1-receptor · Islet transplantation · Glucose oxidation · Diabetes

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Abbreviations: Ang II, Angiotensin II · AT_1, angiotensin II receptor type 1 · AT_2, angiotensin II receptor type 2 · Ao, angiotensinogen · RAS, Renin-angiotensin system · KRBB, Krebs-Ringer bicarbonate buffer

The renin-angiotensin system (RAS) has long been known best for its haemodynamic regulation by means of two major angiotensin II (Ang II) receptors, AT_1 and AT_2 [1]. The two definitive enzymes for this system are renin and angiotensin-converting enzyme (ACE), which determine the generation of the physiologically active peptide, Ang II. The biosynthetic pathways for this classic cascade to produce Ang II and several bioactive peptides such as Ang III, Ang IV and Ang (1–7) are schematically illustrated in Fig. 1. However, alternate enzymes to renin and ACE including kallikrein, tonin and chymase can generate Ang II directly from its precursor angiotensinogen

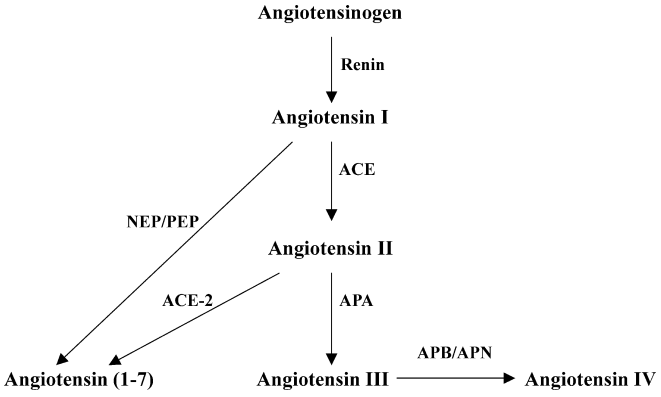

Fig. 1. Outline of the biosynthetic pathways of the renin-angiotensin system. In this system, the precursor angiotensinogen is an obligatory component while renin and ACE are two important enzymes for the cascade. ACE, angiotensin-converting enzyme; ACE-2, a homologue of ACE; APB, aminopeptidase B; APN, aminopeptidase N; NEP, neutral endopeptidase; PEP, propylendopeptidase

(Ao) rather than the definitive enzymatic cascade of Ang II generation [2]. It is therefore more appropriate to describe the RAS as an "angiotensin-generating system", which consists of obligatory components (Ao, Ang II and its receptors), linked by non-exclusive components (renin and ACE).

In recent years, the existence of a local angiotensin-generating system in multiple tissue organs has been shown [3, 4]. This implies that locally produced Ang II exerts local actions from such diverse targets as the heart [5], adrenals [6] and gonads [7] to the pancreas, recently reviewed [8]. Previous studies have shown the presence of such a local system in the pancreas of various species, including the dog [9], rat [10, 11], mouse [12] and human [13]. Available data suggest some potential roles for the angiotensin-generating system in the exocrine and endocrine pancreas [14], such as regulation of ductal anion secretion in the exocrine pancreas [15]. Nevertheless, the potential existence and role of an angiotensin-generating system in the pancreatic islets remain largely unexplored. Indications for a role of Ang II in islets were provided by a perfusion study of whole pancreata, whereby the first phase of glucose-stimulated insulin release was markedly impaired by Ang II [16]. However, this finding was solely attributable to the potent vasoconstrictor action of Ang II, which caused a decrease of islet blood flow in the pancreas.

The present study, therefore, aimed specifically to investigate the existence of an angiotensin-generating system in the pancreatic islets; if so, to elucidate its role in the regulation of insulin secretion and its biosynthesis by studies of isolated pancreatic islets. On the other hand, components of the angiotensin-generating system in the pancreas have previously been shown to be up-regulated by chronic hypoxia [17, 18]. In view of the sustained decrease of oxygen tension in pancreatic islet transplants [19, 20, 21], we also investigated the expression of angiotensin-generating components in retrieved transplanted islets.

Materials and methods

Animals. Male C57BL/6J mice were used in all experiments. The animals were provided by the Laboratory Animal Services Centre of the Chinese University of Hong Kong. The experimental procedures were approved by the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong and followed the "Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985).

Pancreatic islet isolation. Islets were isolated from the pancreas as described previously [22]. Briefly, C57BL/6J mice aged 10 weeks were killed by cervical dislocation and the pancreata were dissected out. The pancreata were then placed in cold Hanks' solution (Sigma Aldrich, St. Louis, USA) and injected with Hanks' solution by means of a 27 G syringe. The inflated pancreata were cut into small pieces with similar size. These pieces were transferred to vials containing collagenase solution (25 mg/8 ml Hanks) (Roche Molecular Biochemicals, Mannheim, Germany). The vials were shaken vigorously at 37°C for approximately 15 min. The collagenase digestion was terminated by the addition of cold Hanks' solution and the digest was then washed three times by filling the vial with Hanks. The islets were then picked using mouth-pipettes and cultured free-floating for 4 to 7 days in non-adherent culture dishes using RPMI 1640 medium (Sigma Aldrich, St Louis, USA) supplemented with 10% (vol/vol) fetal bovine serum (Sigma Aldrich). Medium was changed every other day.

Measurements of insulin release. Groups of ten islets were transferred in duplicate to Falcon 24-well culture plates containing 0.25 ml Krebs-Ringer bicarbonate buffer (KRBB) supplemented with 10 mmol/l HEPES and 2 mg/ml of BSA. For the first hour of incubation at 37°C (O_2/CO_2, 95:5), the medium contained 1.7 mmol/l glucose. The medium was then removed and replaced with 0.25 ml of KRBB containing 16.7 mmol/l glucose and incubated for another hour [23]. The effect of Ang II (Sigma Aldrich) on insulin release from isolated islets was determined at 0.1, 1, 10 and 100 nmol/l of Ang II. Ang II was added in the KRBB medium with 16.7 mmol/l glucose during the second hour of incubation. Specific Ang II-receptor antagonists for AT_1, losartan (Merck & Co., N.J., USA; 1 μmol/l) and for AT_2, PD123319 (Sigma, St. Louis, Mo., USA; 1 μmol/l) were administered 10 min before the Ang II treatment. After the incubation, the medium was collected for the measurement of insulin release using a mouse insulin ELISA kit (Mercodia, Uppsala, Sweden).

Measurements of islet glucose oxidation rate. Triplicate groups of ten islets were transferred to glass vials containing 100 μl of KRBB supplemented with D-[U-^{14}C]glucose (Amersham-Pharmacia Biotech, Uppsala, Sweden) and non-radioactive glucose to a final glucose concentration of 1.7 or 16.7 mmol/l glucose, respectively (spec. act. 0.5 mCi/mmol). To some vials Ang II was added to a final concentration of 100 nmol/l. The islet glucose oxidation rates during 90-min incubation at 37°C (95%O_2/5%CO_2) were measured as described elsewhere [24].

Measurements of (pro)insulin and total protein biosynthesis. Duplicate groups of ten islets were incubated, with or without 100 nmol/l Ang II, at 37°C in 100 μl of KRBB containing

Table 1. Sequence of specific PCR primers and fluorescent probes for the RAS components, and *β-actin* used for the real-time quantitative RT-PCR. *Ao* denotes angiotensinogen

RAS gene	Sequence	Corresponding sequence of GeneBank
AT_1	Forward: CCA TTG TCC ACC CGA TGA AG	458–477 (s37484)
	Reverse: TGC AGG TGA CTT TGG CCAC	520–502 (s37484)
	Probe: CTC GCC TCC GCC GCA CGA	479–497 (s37484)
AT_2	Forward: CAG CAG CCG TCC TTT TGA TAA	176–196 (U04828)
	Reverse: TTA TCT GAT GGT TTG TGT GAG CAA	256–233 (U04828)
	Probe: CAA CTG GCA CCA ATG AGT CCG CCT	205–228 (U04828)
ACE	Forward: TGA GAA AAG CAC GGA GGT ATC C	336–357 (M55333)
	Reverse: AGA GTT TTG AAA GTT GCT CAC ATC A	420–396 (M55333)
	Probe: ACC CTG AAA TAT GGC ACC CGG GC	364–386 (M55333)
Ao	Forward: GAG GCA AAT CTG AGC AAC ATT G	1196–1216 (NM_007428)
	Reverse: GAG TTC GAG GAG GAT GCT ATT GA	1269–1247 (NM_007428)
	Probe: TGA CAC CAA CCC CCG AGT GGG A	1218–1239 (NM_007428)
β-actin	Forward: CCG TGA AAA GAT GAC CCA GAT C	267–288 (M12481)
	Reverse: CAC AGC CTG GAT GGC TAC GT	339–320 (M12481)
	Probe: TGA GAC CTT CAA CAC CCC AGC CAT G	294–318 (M12481)

50 μCi/ml of L-[4,5–3H]-leucine (Amersham-Pharmacia) and 1.7 or 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 measured by an immunoabsorption technique [25], and the total protein biosynthesis was measured in trichloroacetic acid precipitates of the islet homogenate.

Pancreatic islet transplantation. The method for islet transplantation has been described previously [19, 20, 21, 26]. Briefly, three hundred isolated islets were packed into the tip of the mouth-pipette after 4 to 7 days of culture. A recipient C57BL/6J mouse was anaesthetised with an i.p. injection of 2.5% (wt/vol) pentobarbital and a small incision was made on the left renal capsule. The tip of the mouth-pipette was introduced between the renal parenchyma and the capsule followed by gentle ejection of the pellet. Four weeks after transplantation, the mice were killed and the islet transplant was dissected out. As control, pancreatic islets isolated from animals matched for age were used.

Real-time RT-PCR analysis. Real-time quantitative RT-PCR was carried out using an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Foster City, Calif., USA). Total RNA was extracted from pools of all islets isolated from control and retrieved from transplanted mice (groups of 8–10 mice each), using the TRIzol reagent kit (Life Technologies, Gaithersburg, Md., USA) according to the manufacturer's instructions. Total RNA was also extracted from liver and kidney to study the relative abundance of islet mRNA compared to these tissues. The RNA was used as template in one-step Taq Man amplification reactions. TaqMan primers and probes for *Ao*, *ACE*, AT_1 and AT_2 were designed from mouse cDNA sequence using Primer Express Software purchased from Applied Biosystems Perkin-Elmer. Mouse β-actin RNA was used as internal control. TaqMan reactions were set up in a reaction volume of 25 μl by using components supplied in a Taq Man PCR reagent kit. Each reaction consisted of 12.5 μl PCR master mix, 18 μmole of each amplification primer, 25 μmol/l corresponding Taq Man probe and 30 ng RNA template. Each sample was run in duplicate with initial 30 min at 48°C and 10 min at 95°C for reverse transcription, followed by 40 cycles at 95°C for 15 s and at 60°C for 1 min. Amplification data were collected by the 7700 Sequence Detector and analysed by using the Sequence Detection System software. The RNA concentration was determined from the threshold cycle (C_T) at which fluorescence is first detected, the cycle number being inversely related to RNA concentration. The brief details of RAS sequence of PCR primers and their specific probes, as well as corresponding cDNA sequence, were listed in Table 1. The fold changes in mRNA for RAS by islet transplantation were calculated using the $2^{-\Delta\Delta CT}$ method, as described in the section of statistical data analysis.

Western blot analysis. Islets were isolated from control or retrieved from transplanted mice. Protein of 300 islets was extracted using CytoBuster Protein Extraction Reagent (Novagen, Darmstadt, Germany). Protein content of lysates was determined by a Bio-Rad protein assay kit (Bio-Rad, Munich, Germany). Western blot analysis of AT_1-receptor protein expression was done according to our previously described protocol [27]. Briefly, proteins (10 μg/lane) were subject to electrophoresis on a 12% (wt/vol) polyacrylamide. The blotted protein was saturated with 5% (wt/vol) of skimmed milk in phospate-buffered saline (PBS; pH 7.4) and 0.1% (vol/vol) of Tween 20 for 1 h at room temperature. The membrane was sequentially incubated in rabbit anti-AT_1 serum (1:300 dilution) overnight at 4°C and a peroxidase-labelled anti-rabbit IgG antibody (1:1300 dilution) for 1 h at room temperature. The positive signal was revealed using ECL plus western blotting detection reagents and autoradiography film (Amersham, Buckinghamshire, UK). The chemiluminescence intensity of the bands was quantified using an image analyser (Molecular Dynamics Image Quant, Sunnyvale, Calif., USA).

Immunohistochemical localization of AT1 receptor. Immunohistochemistry coupled with a double-staining technique [28, 29] was used to determine the specific localisation of AT_1-receptor in islets. Isolated islets were fixed with 4% (vol/vol) cold paraformaldehyde and embedded in paraffin. Sections (5 μm thick) were mounted on glass slides, deparaffinized and processed for indirect immunofluorescent double staining. After several washes with PBS (pH 7.4), each slide was incubat-

ed with 4% (wt/vol) Normal Donkey serum (Jackson ImmunoResearch, Pa., USA) for 60 min at 37°C. Excess blocking solution was removed and the slide was incubated overnight at 4°C with rabbit anti-AT_1 serum (Santa Cruz Biotech., Santa Cruz, Calif., USA), diluted to 1:25, and with goat anti-insulin serum (Santa Cruz Biotech.), diluted to 1:50. After three washes with PBS, the primary antibodies were detected using an anti-rabbit antibody labelled with Rhodamine (AT_1-receptor) and an anti-goat antibody labelled with Aminomethylcoumarin Acetate (insulin) (Jackson ImmunoResearch) at room temperature for 1 h. Preadsorption and omission of primary antibodies were used as negative controls. Positive immunostaining for AT_1 (red) and for insulin (blue) was examined with a fluorescent microscope equipped with a DC 200 digital camera (Leica Microsystems).

Statistical data analysis. Results were expressed as means ± SEM for all groups. Multiple comparisons between data were done using ANOVA followed by Tukey's test, or, when comparisons only were made toward control, by Dunnett's test. When only two groups were compared, probabilities (p) of chance differences between the experimental groups were calculated with Student's unpaired two-tailed t test. For all comparisons, $p<0.05$ was considered statistically significant. For Western blot analysis, data were normalised as a percentage of control. For real-time RT-PCR, the relative expression was normalised as percentage of *β-actin* and calculated using the comparative C_T method of $2^{-\Delta\Delta CT}$, as described previously [30, 31].

Results

Ang II and insulin release from isolated islets. Insulin release from the isolated islets was markedly enhanced as expected when changing glucose concentration in the incubation medium from 1.7 to 16.7 mmol/l (Fig. 2a). However, the glucose-stimulated insulin release was dose-dependently decreased by adding 0.1, 1, 10 and 100 nmol/l of Ang II (Fig. 2a). At the highest concentration of Ang II used (100 nmol/l), the glucose-induced insulin release could be completely prevented. Pretreatment of isolated islets with 1 μmol/l of losartan, a specific antagonist for the AT_1-receptor, before adding Ang II (100 nmol/l) completely restored the insulin secretion to the glucose-stimulated level (Fig. 2b). However, pretreatment with 1 μmol/l of PD123319, a specific antagonist for the AT_2-receptor had no effect on glucose-stimulated insulin release. In contrast, losartan per se had no effect on glucose-stimulated insulin secretion.

Ang II and islet glucose oxidation rate, (pro)insulin and total protein biosynthesis. Islet glucose oxidation rate, (pro)insulin and total protein biosynthesis were, as the insulin release, markedly higher at 16.7 mmol/l than 1.7 mmol/l glucose (Fig. 3 and Fig. 4). Addition of Ang II (100 nmol/l) to the islets during incubation did not affect either the islet glucose oxidation rate at 1.7 or 16.7 mmol/l glucose (Fig. 3). Islet (pro)insulin biosynthesis at 16.7 mmol/l glucose but not at 1.7 mmol/l glu-

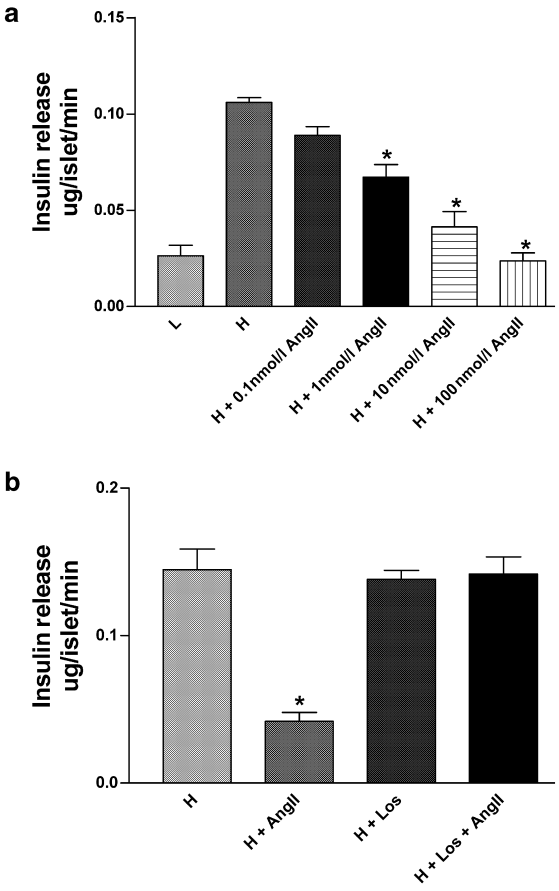

Fig. 2. (**a**) Insulin release from isolated mouse islets in the presence of 1.7 (low; L) or 16.7 mmol/l (high; H) glucose. Ang II was applied at concentrations of 0.1, 1, 10 and 100 nmol/l at the higher glucose concentration. (**b**) Effects of losartan (Los, 1 μmol/l) and Ang II (100 nmol/l) on the glucose (16.7 mmol/l)-stimulated insulin release from isolated islets. All data are expressed as means ± SEM for four experiments in each group. *denotes $p<0.05$ when compared to islets exposed to 16.7 mmol/l glucose only

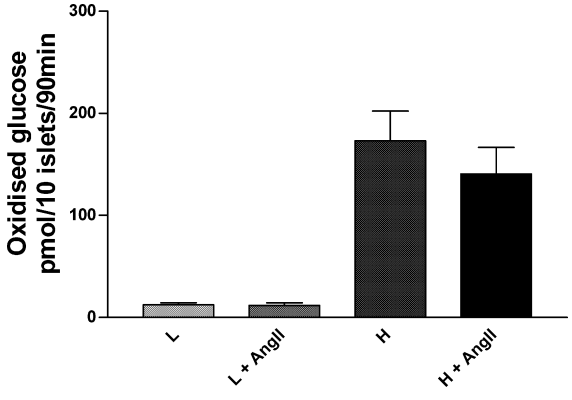

Fig. 3. Influence of Ang II (100 nmol/l) on the glucose oxidation rate in isolated mouse islets exposed to 1.7 (low; L) or 16.7 mmol/l (high; H) glucose. All data are expressed as means ± SEM for nine experiments in each group

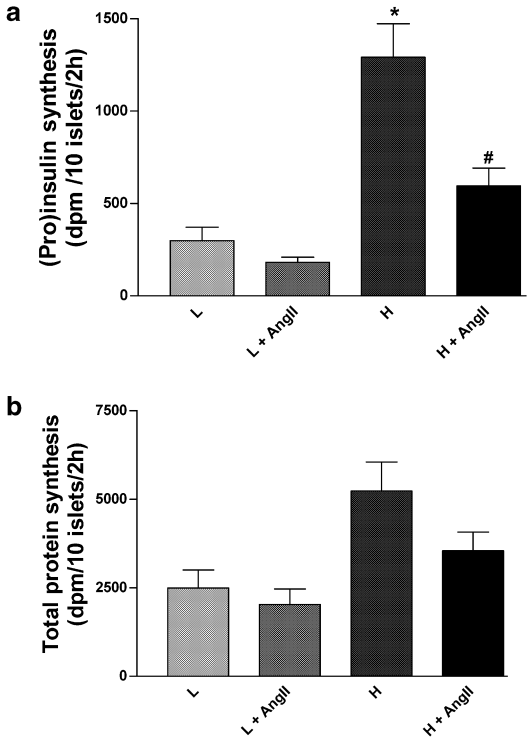

Fig. 4. Influence of Ang II (100 nmol/l) on the (pro)insulin (**a**) and total protein (**b**) biosynthesis in isolated mouse islets exposed to 1.7 or 16.7 mmol/l glucose. All data are expressed as means ± SEM for six experiments in each group. * denotes $p<0.05$ when compared to corresponding islets exposed to 1.7 mmol/l glucose, whereas # denotes $p<0.05$ when compared to islets exposed to 16.7 mmol/l glucose only

cose was, however, inhibited by 100 nmol/l Ang II (Fig. 4a). Islet total protein synthesis was not affected by Ang II either at 1.7 or 16.7 mmol/l glucose (Fig. 4b).

Expression of RAS components in endogenous and transplanted islets. The expression of several major components, namely AT_1 - and AT_2 -receptors, *ACE* and *Ao*, were examined and compared using real-time quantitative RT-PCR coupled with the $2^{-\Delta\Delta CT}$ comparative method in endogenous and transplanted islets. mRNAs for all the components studied were expressed in endogenous islets (Fig. 5). When compared to endogenous islets, the relative expression of mRNAs for AT_1 to *β-actin* was increased by about threefold in transplanted islets (Fig. 5a). In contrast, the relative expression of mRNAs for AT_2 to *β-actin* was down-regulated to approximately one third in transplanted islets (Fig. 5b). The expression of mRNA for *ACE* in endogenous islets did not differ significantly from transplanted islets (Fig. 5c) whereas the expression of angiotensinogen mRNA in transplanted islets was lower than in endogenous islets (Fig. 5d). As calculated from the C_T (Table 2), the relative abundance of different mRNA studied was 0.9, 1.1 and 1.2 for AT_2, *Ao* and *ACE*, respectively as compared to AT_1 expression being as 1. On the other hand, the relative abundance of AT_1 and AT_2 expression in the mouse islets was about 70%, when compared with that in the liver and kidney based on the calculation from the C_T using *β-actin* as internal control.

Expression of AT1-receptor protein in endogenous and transplanted islets. Western blot analysis was carried out to confirm that findings of AT_1-receptor mRNA in endogenous and transplanted islets were reflected also at the protein level (Fig. 6). A major protein band of about 50 M_r was expressed both in endogenous and transplanted islets; in the latter case this was a band of strong intensity (Fig. 6a). This molecular mass (50 M_r) was in close agreement with that of AT_1-receptor protein in mouse kidney, which was used as a

Table 2. Data on the expression of RAS components in endogenous and transplanted pancreatic islets using the comparative C_T method

	RAS gene	C_T [a]	ΔC_T [b]	$\Delta\Delta C_T$ [c]	Expression relative to control[d]
Transplanted	*β-actin*	16.72 ±0.31			
	AT_1	23.82±0.66	7.1±0.75	−1.47±0.72	2.77
	AT_2	29.96±0.60	13.24±0.64	+1.70±1.20	0.31
	ACE	22.34±0.47	5.62±0.59	+0.94±0.88	0.52
	Ao	26.05±0.50	9.33±0.56	+1.36±0.66	0.39
Control	*β-actin*	15.00±0.62			
	AT_1	23.58±0.82	8.57±0.29		
	AT_2	26.54±0.30	11.54±0.80		
	ACE	19.69±0.60	4.69±0.75		
	Ao	22.98±0.50	7.97±0.15		

Ao Angiotensinogen
[a] The average of the C_T data for each sample
[b] The ΔC_T value is calculated by the subtraction of the β-actin C_T from each sample C_T
[c] The $\Delta\Delta C_T$ value is calculated by subtraction of the control ΔC_T from each transplanted sample ΔC_T
[d] The expression relative to control is calculated using the equation $2^{-\Delta\Delta CT}$

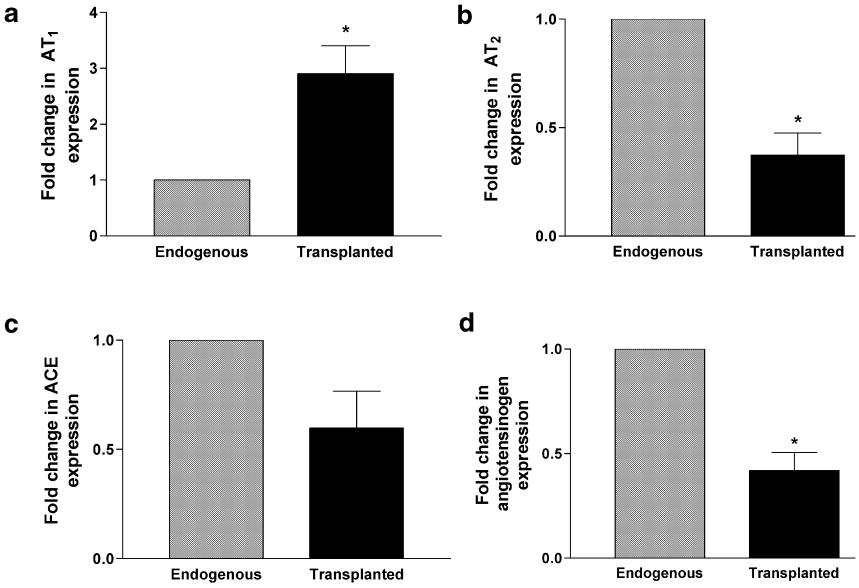

Fig. 5a–d. Real-time RT-PCR analysis of the mRNA expression of (**a**) *AT1* receptor; (**b**) *AT2* receptor; (**c**) *ACE* and (**d**) angiotensinogen in endogenous and transplanted mouse islets. The relative expression was normalised as percentage of *β-actin* calculated using the comparative C_T method of $2^{-\Delta\Delta CT}$ (Table 2). All data are expressed as means ± SEM for four experiments in each group. * denotes $p<0.05$ when compared to endogenous islets

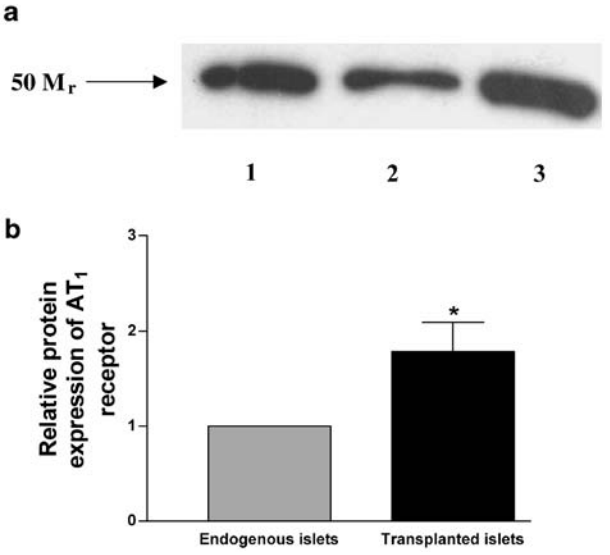

Fig. 6a, b. Western blot analysis of AT_1 receptor protein from endogenous and transplanted mouse pancreatic islets. (**a**) Representative gel image. Lane 1 shows protein of the AT_1 receptor from mouse kidney. A major band of about 50 M_r was detected. Lanes 2 and 3 show the expression of the AT_1 receptor in protein of 10 µg from isolated endogenous and transplanted islets, respectively. (**b**) The relative expression of the AT_1 receptor protein in transplanted islets when compared to endogenous islets. All data are expressed as means ± SEM for four experiments. * denotes $p<0.05$ when compared to endogenous islets

positive control for the expression of the AT_1-receptor. The relative expression of AT_1-receptor protein in transplanted islets was up-regulated twofold when compared to that in endogenous islets, as determined by image analysis (Fig. 6b).

Localization of AT1 receptors in isolated pancreatic islets. A technique with double immunostaining was used for precise localisation of AT_1-receptors in the pancreatic islets (Fig. 7). The localisation of AT_1-receptors (Fig. 7a) to the insulin secreting beta cells (Fig. 7b) was identified by an overlay of immunoreactivity for insulin and the AT_1-receptor (Fig. 7c). Specificity of the immunoreactivity was validated by control experiments either in preadsorption of AT_1 receptor with its blocking peptide in excess (Fig. 7d) or in omission of primary antibody for the AT_1-receptor.

Discussion

Our study provides evidence for the existence of an angiotensin-generating system in the islets by showing the expression of several components (*AT1* and *AT2* receptors, *ACE*, and *Ao*) in the pancreatic islets. Of particular importance in this context is the presence of *Ao*, which constitutes a mandatory component for a local angiotensin-generating system. The existence of such a system makes a local production of Ang II probable, which produces its concentrations several times higher than those measured in peripheral blood. Indeed, islet blood flow seems to be suppressed by such locally produced Ang II during normal conditions [8, 16]. More importantly, Ang II induced a marked and dose-dependent decrease of glucose-stimulated insulin release from isolated islets in the present study, indicating that Ang II plays an important role in regulating islet insulin secretion. With the highest dose of Ang II used (100 nmol/l), the glucose-

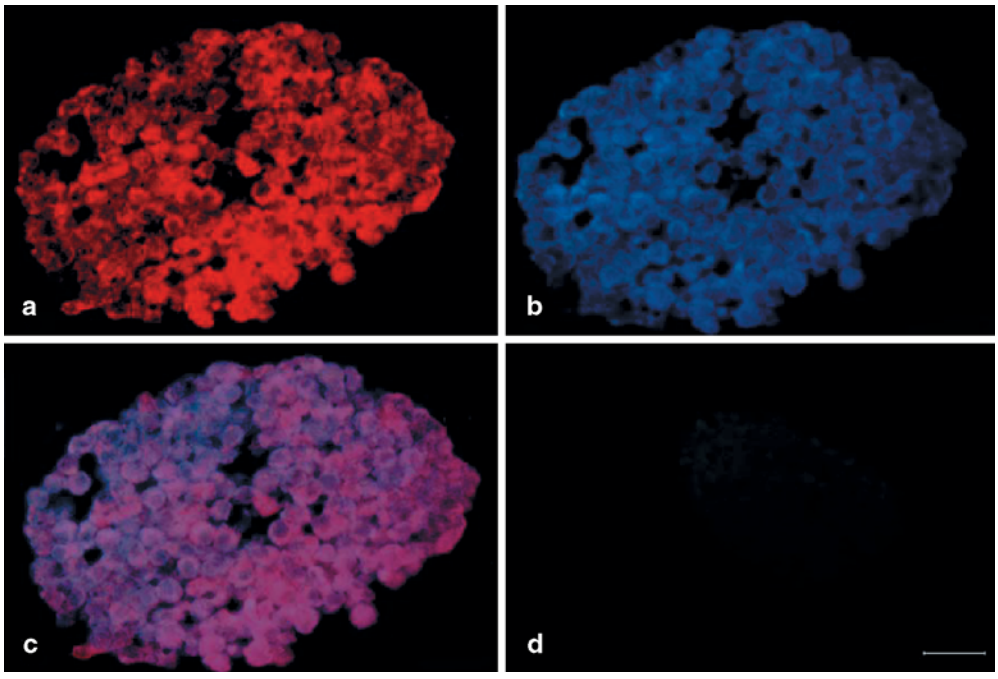

Fig. 7a–d. Immunohistochemical localisation of AT_1 receptors and insulin in isolated mouse pancreatic islets. (**a**) Isolated islet stained for AT_1 receptors (*red*). (**b**) Islet stained for insulin (*blue*). (**c**) Immunoreactivity for that AT_1 receptor was co-localised with that for insulin in the islet beta-cells (*purple*). (**d**) Negative control with the preadsorption of the primary antibodies with its excess blocking peptide. Magnification: 40×. *Bar*=40 μm

stimulated insulin secretion was completely abolished. However, this effect could be fully restored by pretreatment of the islets with the AT_1-receptor antagonist, losartan. In addition, the AT_1-receptors were specifically localised to the islet beta cells as evidenced by double immunofluorescence staining. This further indicates that the inhibitory action of Ang II was mediated through the AT_1-receptors, which were located in the beta cells of pancreatic islets. Previous studies reported that receptors for Ang II have also been identified in the animal pancreatic islets [8, 32], but at least in rat, they were localised preferentially to the surface of alpha- and delta-cells [32]. In the human pancreas positive cells for AT_1-receptor seem, however, to be distributed to those cells stained with insulin [13] although the precise localisation of the AT_1-receptors has yet to be established.

In contrast to the marked effects of Ang II on insulin release from mouse pancreatic islets that we observed, exposure of the rat pancreatic islets to Ang II at a similar dose showed no effects on insulin release in a previous study [33]. This discrepancy of results could be due not only to the species difference but also to the different techniques used in these studies. In this respect, we examined the influence of Ang II on glucose-stimulated (16.7 mmol/l glucose) insulin release, whereas the effect of Ang II on insulin release was studied at low glucose concentration (5.6 mmol/l) in a previous study [33]. The currently employed approach with studies of isolated islets excluded that the inhibitory action of Ang II on insulin release was solely attributable to its vasoconstrictor actions in the pancreatic islets [16]. The inhibitory effect of Ang II on glucose-stimulated insulin secretion from isolated islets was due, at least partly, to the decrease in (pro)insulin biosynthesis. In contrast, islet glucose oxidation rate was unaffected by Ang II, indicating that the suppressive action of Ang II on glucose-stimulated insulin secretion appears not to be the changes in glucose oxidation. Ang II-receptors could influence prostaglandin synthesis, which in turn could modulate insulin secretion [34, 35]. Whether the inhibitory action of Ang II on insulin release from isolated islets is also mediated by this mechanism remains to be determined.

Most interestingly, a decreased incidence of Type 2 diabetes mellitus has been reported in several large clinical studies of patients at high risk of cardiovascular events treated with either ACE-inhibitors [36, 37] or Ang II-receptor antagonists [38, 39]. The exact mechanism(s) for this protection by these RAS blockers against diabetic patients remains equivocal. However, these drugs have been shown in some studies to improve the secretion and action of insulin. Although the effects of ACE-inhibition on insulin sensitivity are not clear, the beneficial effects of ACE-inhibition for insulin release have been shown in many hypertensive patients [40, 41, 42]. Moreover, intravenous infusion of Ang II has suppressed both basal and especially glucose-stimulated insulin secretion in humans [43]. Based on our findings, it is plausible to

speculate that Ang II induced a dose-dependent inhibition of glucose-stimulated insulin release; this could be due to a decrease in (pro)insulin biosynthesis, which was mediated through the AT_1-receptors located in islet beta cells. On the other hand, losartan per se had no effects on glucose-stimulated insulin release from isolated islets in our study. It is not known whether the islet angiotensin-generating system can change or become up-regulated during some disease states, e.g. Type 2 diabetes and hypertension. In such cases, the potential involvement of Ang II and its new regulatory pathway in islet insulin secretion may clarify the mechanism of RAS inhibition for the protection of Type 2 diabetes.

Islet-cell transplantation provides a possibility to accomplish permanent normoglycaemia in patients with Type 1 diabetes mellitus [44]. However, its clinical application is severely restricted by the requirement of considerable number of islet cells [45, 46], which might be due partly to a lack of proper engraftment thus leading to early graft failure [47]. A chronically low oxygen tension and a markedly decreased blood perfusion have been observed in transplanted islets [19, 20, 21]. Both chronic hypoxia and inflammatory conditions have activated and up-regulated RAS components in the pancreas [17, 27, 48]. In the present study, the expression of AT_1 -receptors, in contrast to the expression of AT_2 -receptors, A*CE* and *Ao*, was found markedly up-regulated both at the mRNA and protein levels in transplanted islets, as shown by real-time RT-PCR and western blot analysis, respectively. This observation is consistent with the enhanced vascular sensitivity to Ang II, when compared to endogenous islets, as recorded in islet transplants [49].

In conclusion, the study gives evidence for the presence of a local angiotensin-generating system in the islets. This system provides an inhibitory role for locally produced Ang II of glucose-stimulated insulin secretion, an effect mediated by the AT_1-receptors located on the surface of the islet beta cells.

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