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Down-regulation of aortic and cardiac AT_1 receptor gene expression in transgenic (mRen-2) 27 rats

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1 Transgenic(TG) (mRen-2) rats overexpressing the mouse renin gene develop fulminant hypertension and cardiac hypertrophy. Since the activation of AT_1 receptor by angiotensin II is involved in blood pressure regulation, cardiac performance and myocardial growth, we investigated the biological effects of angiotensin II and the regulation of the AT_1 receptor in the heart and aorta of TGR (mRen-2)27 rats in comparison to control animals.

2 Contraction studies on isolated cardiac muscle strips reveal that angiotensin II exerts no positive inotropic effect on the left ventricular myocardium of both, transgenic and control rats. In contrast, angiotensin II leads via AT_1 receptor activation in the left atrium of control rats to a significant contraction ($130 \pm 5\%$ of basal contraction) which is not detectable in left atrium preparations of the transgenic animals. Furthermore, AT_1 receptor activation causes a profound contraction of aortic rings isolated from control rats amounting to 1.39 ± 0.2 mN mg^{-1} wet weight, whereas aortic rings from TGR (mRen-2)27 rats contract only minimally upon angiotensin II stimulation (0.2 ± 0.02 mN mg^{-1} wet weight).

3 These altered physiological responses of angiotensin II in the transgenic rats are in part due to a marked down-regulation of the AT_1 receptor in atrial, ventricular and aortic tissue of these transgenic animals in comparison to control Sprague-Dawley rats, as shown by radioligand binding assays and quantitative polymerase chain reaction (PCR) experiments. The AT_1 receptor density B_{max} in the left atrium was 1.3 ± 0.08 fmol mg^{-1} protein in control rats (K_D 1.1 ± 0.18 nmol l^{-1}) and 0.94 ± 0.15 fmol mg^{-1} protein (K_D 2.1 ± 0.3 nmol l^{-1}. In the aorta B_{max} values were 15.1 ± 0.5 fmol mg^{-1} protein (K_D 1.9 ± 0.27 nmol l^{-1}) for control rats and 11.3 ± 0.76 fmol mg^{-1} protein (K_D 1.9 ± 0.27 nmol l^{-1}) for the TGR(mRen-2)27 rats AT_1 receptor mRNA was reduced in the transgenic animals to $46 \pm 3\%$ in the left atrium, $50 \pm 11\%$ in the left ventricle and $40 \pm 3\%$ in the aorta, respectively.

4 Together, the AT_1 receptor is down-regulated in TGR (mRen-2)27 rats in comparison to wildtype Sprague Dawley rats leading to a profoundly decreased response of cardiac and aortic tissue upon stimulation with angiotensin II.

Keywords: Angiotensin II receptor; hypertension; cardiac hypertrophy; transgenes; renin AT_1 receptor

Introduction

The renin-angiotensin system plays an important role in the development of hypertension (Caponi *et al*., 1981). In an effort to elucidate the pathogenesis of this disease, the transgenic (TG)(mRen-2)27 rat, in which the Ren-2 mouse renin gene is introduced into the genome of the Sprague-Dawley (SD) rat was established. These animals develop severe hypertension at a young age. The transgene is expressed at high levels in the adrenal, thymus, small intestine, testis, ovary, kidney, brain, lung, blood vessels, pituitary, thyroid and myocardium (Mullins *et al*., 1990; Ganten *et al*., 1991). TG(mRen-2)27 rats have elevated plasma prorenin levels, but, surprisingly, the serum levels of renin and angiotensin II were normal in heterozygous animals (Mullins *et al*., 1990). In contrast, a recent study on homozygous transgenic animals provided evidence that renin as well as angiotensin II plasma levels are increased (Hilgers *et al*., 1992). Nevertheless, the fulminant hypertension of the TG(mRen-2)27 rats respond to treatment with angiotensin-converting enzyme (ACE) inhibitors as well as AT_1 receptor antagonists indicating that angiotensin II is decisively involved in this monogenic form of hypertension (Mullins *et al*., 1990; Ganten *et al*., 1991; Lo *et al*., 1993; Moriguchi *et al*., 1994). Most of the known biological effects of angiotensin II are mediated by the angiotensin II-type 1 (AT_1) receptor. The AT_1 receptor is a G-protein coupled receptor expressed in numerous tissues (Peach, 1977). It is well established that activation of AT_1 receptors expressed on vascular smooth muscle cells leads to vasoconstriction and ultimately to elevated blood pressure. Moreover, AT_1 receptor activation by angiotensin II is thought to be involved in cardiac hypertrophy, whereas the effects of angiotensin II on cardiac contractility remain the subject of ongoing controversy (Khairallah & Kanabus, 1983; Baker & Aceto, 1990; Sadoshima & Izumo, 1993; Holubarsch *et al*., 1993; Ishihata & Endoh, 1993; 1995; Kim *et al*., 1995). As a member of the G-protein coupled receptor family, the AT_1 receptor expression is regulated *in vitro* as well as *in vivo* (Gunther *et al*., 1980; Aguilera & Catt, 1981; Schiffrin *et al*., 1984; Belluci & Wilkes, 1984; Nickenig & Murphy, 1994; Lassegue *et al*., 1995). Since most effects of angiotensin II are mediated by the AT_1 receptor, the regulation of this gene may substantially influence the activity of the entire renin-angiotensin system. TG(Ren-2)27 rats develop severe hypertension and cardiac hypertrophy which are both closely related to the activated renin-angiotensin system (Mullins *et al*., 1990; Ganten *et al*., 1991). Consequently, it is of interest to characterize quantitatively the activation and expression of the AT_1 receptor which are involved in the pathogenesis of hypertension in these transgenic rats. Therefore, we investigated the potentially modulated effects of angiotensin II in TG(Ren-2)27 rats on cardiac and aortic contractility and attempted to correlate

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these (patho-)physiological effects to the level of cardiac and aortic AT_1 receptor expression.

Methods

Transgenic animals

Transgenic animals (TG(mRen-2)27) were housed and bred in the animal laboratory of the Max Delbrück Centrum (MDC) for Molecular Medicine in Berlin. At the age of five weeks, heterozygous male animals were transferred to the animal laboratory of the University of Cologne (Germany). Sprague Dawley (SD) control rats were obtained from the Laboratorium für Versuchstierkunde in Hannover (Germany). This is the strain into which the transgene was originally introduced. The experiments were performed at an age of 12–14 weeks. At this age hypertension is fully established (i.e. TG(mRen-2)27: 230 ± 8 mmHg vs SD: 115 ± 12 mmHg systolic blood pressure, recorded by the tail-cuff method). Rats were killed by a blow on the head and the tissues were rapidly removed and arrested in ice-cold 0.9% NaCl.

Isolated cardiac muscle strip and measurement of force of contraction

Atrial trabeculae obtained from the left atria and papillary muscles were isolated from left ventricles. Immediately after excision, the papillary muscles and atrial trabeculae were placed in ice-cold preaerated Tyrode solution. The experiments were performed on isolated electrically driven muscle preparations. Muscle strips of uniform size with muscle fibres running approximately parallel to the length of the strips were dissected under microscopic control with scissors in aerated modified Tyrode solution at room temperature. Connective tissue was trimmed away carefully. The muscles were suspended in an organ bath (75 ml) maintained at 37°C and containing a modified Tyrode solution of the following composition (in mM): NaCl 119.8, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, Na_2HPO_4 0.42, $NaHCO_3$ 22.6, Na_2EDTA 0.05, ascorbic acid 0.28 and glucose 5.0. The bathing solution was continuously aerated with 95% O_2 and 5% CO_2. The muscles were stimulated by two platinum electrodes (frequency 1 Hz, impulse duration 5 ms; intensity 10–20% greater than threshold) by field stimulation from an electronic stimulator (Grass S88, Quincy, MA, U.S.A.). Each muscle was stretched to the length at which force of contraction was maximal. Isometric force of contraction was measured with an inductive force transducer (W. Fleck, Mainz, Germany) attached to a Gould recorder (Brush 2400, Gould Inc, Cleveland, Ohio, U.S.A.). All preparations were allowed to equilibrate for at least 90 min, with the bathing solution being changed once after 45 min. Concentration-dependent mechanical effects were obtained. Control strips kept in Tyrode solution with identical composition as original experiments revealed maximally 10% reduction of baseline isometric tension over the period necessary to complete pharmacological testing. Agents were applied cumulatively to the organ bath. Each muscle was used only once to record a concentration-response curve.

Aortic ring preparations and tension recording

After excision of the descending aorta, the vessel was immersed in Krebs buffer and adventitial tissue was removed. Ring 2–3 mm long were mounted for recording of isometric tension in organ baths filled with Krebs buffer which was continuously aerated with 95% O_2 and 5% CO_2. The preparations were attached to a force transducer and isometric tension was recorded on a polygraph. Tissues were allowed to equilibrate for 90 min. A resting tension of 2 g was maintained throughout the experiment. Drugs were added in increasing concentrations in order to obtain cumulative concentration-response curves. Angiotensin II was used at a concentration from 1 nmol l^{-1} to 10 μmol l^{-1}. The drug concentration was increased when vasoconstriction was completed which took on average 5–10 min for each step.

Membrane preparation and radioligand binding assays

The aortic and myocardial tissue was chilled in 30 ml ice-cold homogenization buffer (20 mM Tris/HCl, 1 mM EDTA, 1 mM dithiothreitol, pH 8.0). Connective tissue was trimmed away and the tissue was minced with scissors, disrupted with an Ultraturrax (Janke and Kunkel, Staufenbreisgau, Germany) and homogenized with a motor-driven glass teflon Potter for 1 min. The homogenate was spun at 480 *g* for 10 min (JA 20, Beckman, Palo Alto, U.S.A.). The supernatant was diluted with an equal volume of ice-cold 1 M KCl and stored on ice for 10 min and centrifuged at 100,000 *g* for 45 min. The pellet was resuspended in 50 volumes of homogenization buffer and recentrifuged at 100,000 *g* for 45 min. The final pellet was resuspended in an incubation buffer in the absence of DTT (50 mM Tris/HCl, 50 mM NaH_2PO_4, 10 mM $MgCl_2$, 0.2% bovine serum albumin and proteinase inhibitors: trypsin inhibitor 0.2 mg ml^{-1}, pepstatin A 0.25 mg ml^{-1} and leupeptin 0.25 mg ml^{-1}, pH 7.1). Angiotensin II receptors in cardiac and aortic tissue were investigated in saturation experiments with increasing amounts of [^{125}I]-angiotensin II as radiolabelled ligand (0.125–4 nM). Dup753 (10 μM) was used to determine nonspecific binding. The assay was performed in a total volume of 250 μl incubation buffer. The incubation was carried out at 24°C for 60 min. These conditions allowed a complete equilibration of the receptor with the radioligand. The reaction was terminated by rapid vacuum filtration through Whatmann GF/C filters (Whatman, Clifton, New Jersey); the filters were washed immediately three times with 5 ml of ice-cold incubation buffer and radioactivity was determined in a γ-counter. All experiments were performed in triplicate. The maximal density (B_{max}) and apparent affinity (K_D) of binding sites were obtained by non-linear regression analysis.

mRNA isolation and quantitative polymerase chain reaction

Atrial and ventricular myocardium and the abdominal aorta were isolated, quickly frozen in liquid nitrogen and homogenized. RNA was isolated with RNA-clean (AGS, Heidelberg, Germany) according to the manufacturer's protocol in order to obtain total cellular RNA. This was quantified spectrophotometrically by measuring absorbance values at 260 and 280 nm. Two microgram aliquots were electrophoresed through 1.2% agarose-0.67% formaldehyde gels and stained with ethidium bromide to verify the quantity and quality of the RNA. The original AT_1 receptor cDNA (Ca18b, Murphy *et al.*) was digested with MSCI and self ligated. The resulting plasmid lacking the region from base 446 to 734 (mutAT1) was linearized by digestion with SacI and a deletion mutated AT_1 receptor mRNA was *in vitro* transcribed with the Megascript-Kit (Ambion) following the manufacturer's instructions. Two micrograms of the isolated total RNA and 10 pg of the mutAT_1 mRNA were mixed and reverse transcribed with random primers. The single stranded cDNA was amplified by polymerase chain reaction with Taq DNA-polymerase (Boehringer, Mannheim, Germany); 28 cycles were performed under the following conditions: 30 s, 94°C, 55°C, 45 s; 72°C, 45 s. The sequence for AT_1 receptor sense and antisense primers were: 5′-ACCCCTCTACAGCATCTTTGTGGTGGGGA-3′ and 5′-GGGAGCGTCGAATTCCGAGACTCATAATGA-3′, respectively. The same samples were used for GAPDH cDNA amplification to confirm that equal amounts of RNA were reverse transcribed. The primers employed were 5′-ACCACAGTCCATGCCATCAC-3′ and 5′-TCCACCACCCTGTTGCTGTA-3′. PCR amplification gave 479 bp, 191 bp, and 452 bp of fragments originated from the AT_1 receptor mRNA, the mutated AT_1 receptor mRNA and GAPDH mRNA, respectively.

Materials

Angiotensin II (AII, human, acetate salt), phenylephrine (-hydrochloride), isoprenaline (1-[3′,4′-dihydroxyphenyl]-2-isopropylaminoethanol) and KCl were obtained from Sigma (Deideshofen, Germany). DuP 753 (losartan potassium, 2-butyl-4-chloro-5-(hydroxymethyl)-1-[[2″-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]imidazole) was purchased from Merck Sharp and Dohme (Munich, Germany) and PD 123177 (1-4(4-amino-3-methyl-phenyl)methyl-5-diphenylisoethyl-4,5,6,7-terahydro-1H-imidazo[4,5-C]pyridine-6-carboxylic acid-2HC) was from Park Davis (Berlin, Germany). [^{125}I]-angiotensin II and [^{32}P]-dCTP (deoxycytidine-5′[α-^{32}P] triphosphate were obtained from Amersham-Buchler (Braunschweig, Germany). Restriction enzymes and PCR products including Reverse Transcriptase and Taq Polymerase came from Boehringer-Mannheim (Mannheim, Germany). All other compounds used were of analytical or best grade commercially available. Only deionized and distilled water was used throughout.

Statistics

Data are presented as means ± s.e. Statistical analysis was performed by analysis of variance (ANOVA).

Results

In TG(mRen-2)27 compared to SD there was a significant increase of absolute and relative heart weights (TG(mRen-2)27: 1.82 ± 0.03 g, 4.92 ± 0.11 mg g^{-1}; SD: 1.42 ± 0.03 g, 3.78 ± 0.08 mg g^{-1}; $P<0.001$). Hearts of TG(mRen-2)27 exhibited concentric hypertrophy, but no dilatation or any signs of heart failure like excessive scarring. No signs of venous congestion was observed in any other organ.

The effects of angiotensin II on force of contraction in isolated electrically stimulated atrial preparations are demonstrated in Figure 1. In atrial trabeculae of control animals, angiotensin II exerted a concentration-dependent positive inotropic effect which was maximal within 1 min. This positive inotropic effect was not detectable in atrial trabeculae isolated from TG(mRen-2)27 rats. In left ventricular preparations from either control or transgenic animals no such effect on force of contraction could be observed at 30 μM angiotensin II (data not shown). To characterize functionally the positive inotropic effect of angiotensin II observed in atrial myocardium, the effect was investigated in the presence of the selective angiotensin II AT_1 and AT_2 receptor antagonists Dup 753 (Losartan) and PD 123177. There were no direct inotropic effects of these agents before angiotensin II application. The positive inotropic effect of angiotensin II (130 ± 5%) in atrial trabeculae was antagonized by 10 μmol l^{-1} of the angiotensin II AT_1 receptor antagonists DuP 753 (102 ± 3%) but remained unchanged in the presence of 3 μM of the angiotensin II AT_2 receptor antagonist, PD 123177 (129 ± 4%) (Figure 1). The positive inotropic effect of isoprenaline showed no significant difference for either control or transgenic animals (Figure 1).

Organ chamber experiments on isolated aortic rings showed a profound concentration-dependent effect of angiotensin II on aortic contraction in control animals, whereas this angio-

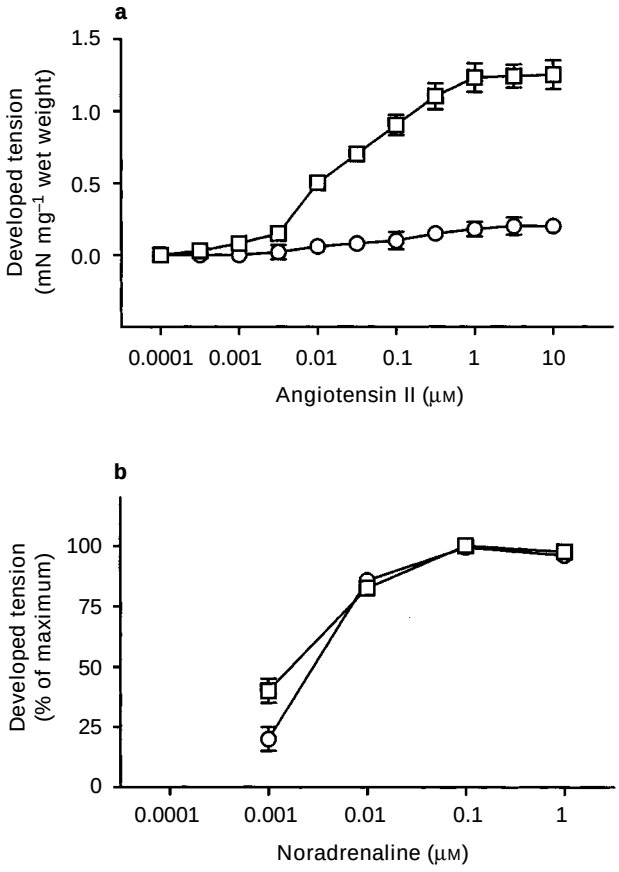

Figure 1 AT_1 receptor mediated force of contraction. Force of contraction as % of basal contraction level in response to either angiotensin II (a) or isoprenaline (b) in left atrial trabeculae isolated from control Sprague-Dawley (□) and TG(mRen-2)27 (○) rats. Each point represents mean data generated from five separate experiments and vertical lines show s.e.

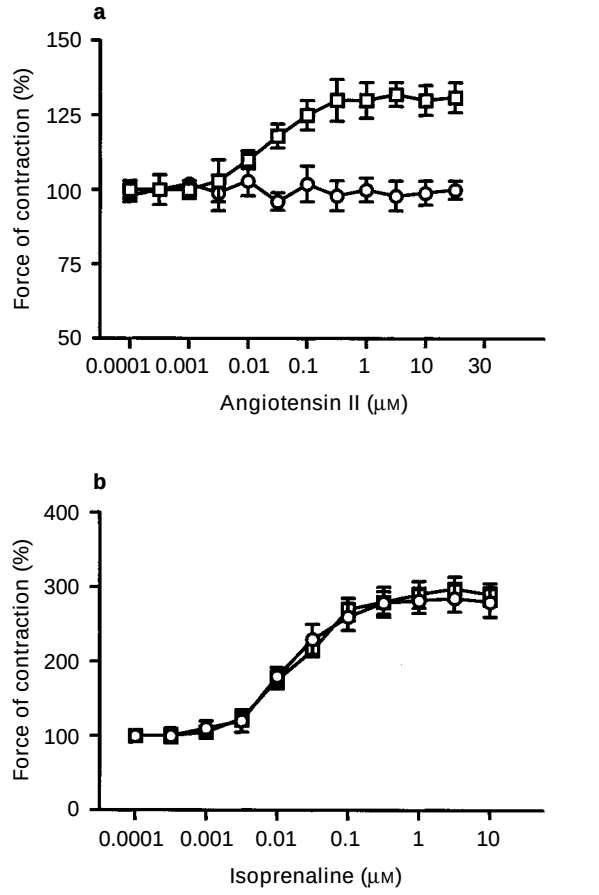

Figure 2 Aortic vasoconstriction. Concentration-response curves for effects of angiotensin II (a) and noradrenaline (b) on contraction of aortic rings isolated from control (□) and TG(mRen-2)27 (○) rats. Force of contraction as % of predrug level (angiotensin II) or as % of maximum constriction in Sprague-Dawley rats (noradrenaline). Each point represents data generated from five separate experiments and vertical lines shown s.e.

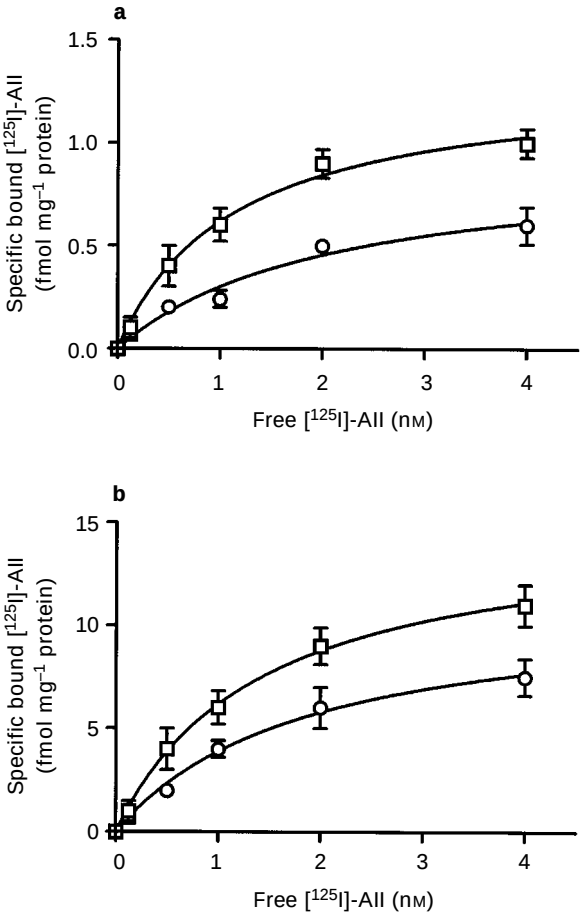

Figure 3 Radioligand binding assays on cardiac and aortic tissue. Saturation binding assay with [^{125}I]-angiotensin II in (a) left atrium and (b) descending aorta isolated from control (□) and TG(mRen-2)27 rats (○). Each point represents mean of three separate experiments; vertical lines show s.e.

tensin II-induced vasoconstriction was only slightly measurable in transgenic animals (Figure 2). The maximal force of contraction amounts in control animals to 1.39 ± 0.2 mN mg^{-1} wet weight whereas in TG(mRen-2)27 rats the maximal tension was measured significantly reduced at 0.20 ± 0.02 mN mg^{-1} wet weight ($P < 0.05$). The $-\log EC_{50}$ for angiotensin II was slightly increased in the transgenic animals (2.0 ± 0.5) in comparison to the SD-rats (1.9 ± 0.2) (NS). Control experiments with potassium chloride demonstrated comparable constrictions in both transgenic and control rats (data not shown). Also shown in Figure 2 is the noradrenaline-induced tension. There was no significant difference between the control and the transgenic rats with respect to EC_{50} values and the maximal force of constriction.

These data demonstrate that the angiotensin II caused positive inotropic and vasoconstrictor effect is markedly and selectively reduced in TG(mRen-2)27 rats in comparison to SD-rats.

In order to investigate the mechanisms underlying this impaired physiological response in TG(mRen-2)27 rats upon angiotensin II stimulation, we quantified the steady state levels of the AT_1 receptor and the AT_1 receptor mRNA. Therefore, radioligand binding assays were performed on left atrial, left ventricular, and aortic tissue. No valid AT_1 receptor binding activity was measurable in the left ventricle, either in Sprague Dawley or in TG(mRen-2)27 rats (data not shown). Figure 3 shows saturation binding assays with [^{125}I]-angiotensin II on left atria and aortae. Analysis of three separate experiments by non-linear regression revealed that the AT_1 receptor density was markedly reduced and the affinity was increased in the left atrium and the aorta of renin-transgenic animals in comparison to control animals (Table 1).

In order to determine the AT_1 receptor mRNA steady state levels we utilized a quantitative PCR approach. The reverse transcription and PCR reaction of the AT_1 receptor mRNA was monitored by including an internal standard. This deletion- mutated AT_1 receptor mRNA yielding a substantially shorter PCR product (191 bp) enabled distinction of the wildtype and mutated AT_1 receptor mRNA (479bp). Quantity

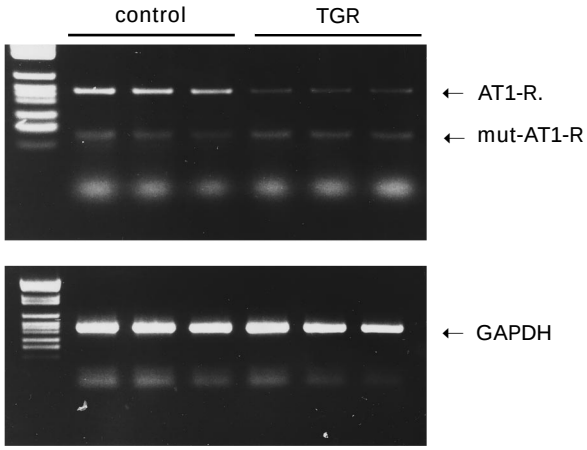

Figure 4 Quantitative AT_1 receptor mRNA PCR. Representative ethidium bromide stained-agarose gel of a reverse transcription-PCR of RNA isolated from aortae excised from either Sprague Dawley or TG(mRen-2)27 rats. The 496 bp DNA fragment corresponds to the AT_1 receptor mRNA, the 191 bp DNA fragment results from the mutated AT_1 receptor mRNA (internal standard). GAPDH mRNA was used as external standard.

Table 1 Calculated data from [^{125}I]-angiotensin II saturation binding assays on left atrium and aorta of Sprague-Dawley and TG (mRen-2)27 rats

	Left atrium		*Aorta*	
	B_{max} *(fmol mg^{-1} protein)*	K_D *(nmol^{-1})*	B_{max} *(fmol mg^{-1} protein)*	K_D *(nmol/l)*
TG(mRen-2)27 rats	0.94 ± 0.15	2.1 ± 0.3	11.3 ± 0.76	1.90 ± 0.27
Sprague-Dawley rats	1.3 ± 0.8	1.1 ± 0.18	15.1 ± 0.5	1.44 ± 0.12

and quality of the included RNA was controlled by an additional PCR reaction from the same reverse transcription samples with an external standard (GAPDH). The exponential phase for the used amounts of wildtype and mutated RNA was found to be in the range 20 and 34 cycles (data not shown). Therefore, 28 cycles were used in our experimental set-up. For quantification of the resulting PCR products, 1 μCi of [^{32}P]-dCTP was included in each reaction and ethidium bromide-stained DNA fragments were excised from agarose gels and counted in a β-counter. Figure 4 shows a representative ethidium bromide-stained gel of the DNA fragments resulting from a RT-PCR of RNA isolated from three aortae of both transgenic and control rats. Whereas PCR products generated from the internal standard and GAPDH mRNA remain at approximately equal levels, the AT_1 receptor mRNA is down-regulated in transgenic animals. Figure 5 illustrates the quantification of AT_1 receptor mRNA calculated to the internal standard in left atrium, left ventricle and the aorta. The relative AT_1 receptor mRNA distribution in left atrium, left ventricle and aorta from SD-rats is illustrated. The AT_1 receptor is predominantly expressed in the aorta rather than in the left atrium. The AT_1 receptor mRNA expression in the left ventricle amounted to approximately 50% of the expression in the left atrium. This relative AT_1 receptor mRNA distribution was similar in TG(mRen-2)27 rats (data not shown). Figure 5 also shows the evaluation of five separate experiments revealing that the AT_1 receptor mRNA is down-regulated in TG(mRen-2)27 rats in comparison to control rats in the left atrial to $46 \pm 3\%$ ($n=5$), in left ventricle to $50 \pm 11\%$ ($n=6$) and in the aorta to $40 \pm 3\%$ ($n=5$), respectively. The external standard GAPDH showed no significant variations.

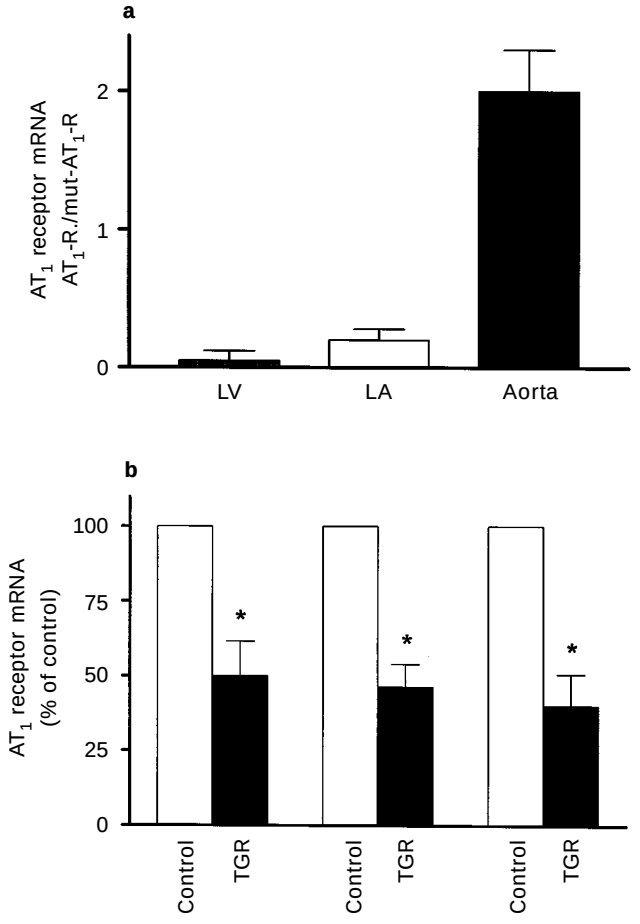

Figure 5 AT_1 receptor mRNA steady state levels. (a) The relative AT_1 receptor expression in left atrium (LA), left ventricle (LV) and aorta is illustrated. The AT_1 receptor mRNA level is expressed in relation to the mutated AT_1-receptor mRNA (mut-AT1-R) which was used as internal standard. (b) AT_1 receptor mRNA steady state levels in TG(mRen-2)27 rats (TGR) in comparison to control rats (as %). Ethidium bromide stained DNA fragments were excised from the gel and the incorporated [^{32}P]-dCTP was quantified in a β-counter and taken as a measure of AT_1 receptor mRNA expression. AT_1 receptor mRNA levels were normalized to the activity of the internal standard. Each point represents data from five separate experiments $\pm$ s.e. $^*P<0.05$.

Discussion

As most of the known biological effects of AII are mediated by the AT_1 receptor subtype (Peach, 1977), regulation of the responsiveness of this receptor has been a prominent subject of recent research. Indeed, it is well established that the AT_1 receptor is regulated *in vivo* as well as *in vitro*. Conditions of increased renin-angiotensin system activity cause down-regulation of AT_1 receptors (Gunther *et al*., 1980; Schiffrin *et al*., 1984), whereas a decrease in the activity of the renin angiotensin system up-regulates the AT_1 receptor (Aguilera & Catt, 1981; Belluci & Wilkes, 1984). Recently, it has been shown that various growth factors as well as AII induce a profound down-regulation of AT_1 receptor gene expression in cultured VSMC (Nickenig & Murphy, 1994; Lassegue *et al*., 1995). Besides this classical vascular angiotensin II receptor physiology, the impact of AT_1 receptors on cardiac function has been evaluated intensively during the past years. AT_1 receptors expressed in the myocardium are thought to mediate inotropic and chronotropic effects. Moreover, angiotensin II-induced activation of the AT_1 receptor leads to enhanced cardiac protein synthesis as well as myocardial hypertrophy (Khairallah & Kanabus, 1983; Baker & Aceto, 1990; Sadoshima & Izumo, 1993; Holubarsch *et al*., 1993; Ishihata & Endoh, 1993; Kim *et al*., 1995; Ishihata & Endoh, 1995). The data generated by Ishihata and coworkers are in part different from our data. They measured angiotensin II receptor expression in rat left ventricles by radioligand binding assays and found a receptor density in the left ventricle of approximately 20 fmol mg^{-1} protein. In contrast, we were not able to detect any AT_1 receptor binding activity in the left ventricle (in SD or in the TG(mRen-2)27 rats) and even in the left atrium (SD rats) we detected only 1.3 fmol mg^{-1} protein. The differences may be due to the different species used (Wistar vs Sprague Dawley rats) and to slightly different membrane preparation and binding conditions. Moreover, Ishihata and coworkers used angiotensin II in their saturation binding experiments in order to define unspecific binding and therefore measured all angiotensin II receptor subtypes and not only the AT_1 receptors.

Although the regulation of the myocardia AT_1 receptor is poorly understood, it has recently been shown that the AT_1 receptor is down-regulated in human failing hearts, suggesting a potentially important involvement of AT_1 receptor regulation in the (patho)-physiology of the heart (Regitz *et al*., 1995). Hypertension and the closely related cardiac hypertrophy substantially participate in the onset and progression of this chronic disease (Spann *et al*., 1967; Grossman *et al*., 1975). In heart failure, neurohormonal systems such as the sympathetic nervous system and the renin-angiotensin system are activated (Kannel *et al*., 1972; Dzau *et al*., 1981). In this context, down regulation of β-adrenoceptors in response to elevated catecholamine serum levels have been obtained (Bristow *et al*., 1982; Brodde, 1987; Böhm *et al*., 1988). However, to date it is still a subject of speculation which mechanisms are precisely involved in the development of heart failure from risk factors such as hypertension and cardiac hypertrophy. The down-regulation of cellular signalling pathways may be involved in this process. Besides the β-adrenergic system, the AT_1 receptor expression in the myocardium could represent a putative candidate.

Recently, transgenic rats overexpressing the renin gene became available. Based on this monogenic variation, the animals develop severe hypertension and cardiac hypertrophy. This model displays an activated renin-angiotensin and adre-

nergic system at is occurs similarly in heart failure (Mullins *et al*., 1990; Ganten *et al*., 1991). The activation of renin-angiotensin system seems to play a key role since treatment with either ACE inhibitors or AT_1 receptor antagonists prevent the progression of cardiovascular disease in this animal model (Mullins *et al*., 1990; Ganten *et al*., 1991; Lo *et al*., 1993; Moriguchi *et al*., 1994). In addition, recent studies have shown blood pressure lowering features of endothelin receptor antagonists in TG(mRen-2)27 rats, although endothelin plasma levels were lower in transgenic versus control rats (Gardiner *et al*., 1995). Interestingly, the authors showed an additive effect, on the lowering of blood pressure, of endothelin receptor and AT_1 antagonists suggesting a role for endothelin in this transgenic animal model. Endothelin is known to be induced by angiotensin II in various tissues (Lüscher, 1994), and therefore the synthesis of endothelin may be enhanced locally by the activated renin-angiotensin system. Alternatively, endothelin and AII may also influence independently the elevation of the blood pressure.

We used this animal model, to elucidate the function and expression of cardiac AT_1 receptors. Interestingly, our data demonstrate that the cardiac and vascular AT_1 receptor expression is down-regulated in transgenic animals. The biological effects of angiotensin II are attenuated in the heart as well as the aorta. Two mechanisms are putatively involved in this down-regulation. First, homologous down-regulation of the AT_1 receptor may be induced by elevated angiotensin II levels generated by the activated renin-angiotensin system. Plasma angiotensin II levels are not increased in the heterozygous transgenic animal but it is very likely that angiotensin II levels are increased locally by the activated tissue renin-angiotensin system (RAS). Second, heterologous down-regulation of the AT_1 receptor could be mediated by, for example, the activated adrenergic system. The AT_1 receptor down-regulation may be considered as a compensatory and protective mechanism in response to the activated RAS. Nevertheless, these data are surprising since the cardiovascular alterations in these animals are obviously caused by an activated RAS despite the down-regulation of the AT_1 receptor meaning that the protective mechanisms initiated by the organism are obviously not sufficient to overcome cardiovascular disease in these animals. The question arises how many AT_1 receptors does the RAS need to cause such profound pathophysiological changes? So far this remains unanswered demonstrating that our understanding of the RAS and its mechanisms of action is still rudimentary. Alternatively, one may speculate that hypertension in the transgenic animals is at the age of 12 weeks already independent of the renin-angiotensin system due to structural changes in the heart and in the vasculature. This notion would be in agreement with a recent study (Arribas *et al*., 1994) showing that the angiotensin II-induced vasoconstriction is enhanced in TG(mRen-2)27 rats at the age of four weeks.

In any event, down-regulation of vascular and cardiac G-protein coupled receptors seems to represent a general mechanism involved in diseases which are associated with increased activity of the renin-angiotensin as well as the adrenergic system. It is attractive to speculate that the destabilization of G-protein coupled receptor mRNA is one fundamental pathway underlying this phenomenon, since inducible degradation of the receptor mRNA is thought to be essential in the regulation of β-adrenoceptors (Hadcock *et al*., 1989), as well as AT_1 receptors (Nickenig & Murphy, 1994; 1996; Lassegue *et al*., 1995).

Further research will be required to elucidate whether this general compensatory receptor down-regulation plays a crucial role in the progression of heart failure. It has to be emphasized that the AT_1 receptor down-regulation described in the present paper probably accounts for only one mechanism involved in the dramatically impaired effects of angiotensin II on myocardial contractility and aortic vasoconstriction. An alternative pathway could be the function uncoupling of AT_1 receptors as suggested by the increase in the ligand affinity in TG(mRen-2)27 rats, although to date no valid data are available to strengthen this notion of intracellular desensitization.

Finally, the effects of angiotensin II on myocardial contractility appear to differ among species. Positive inotropic and chronotropic effects have been obtained in dogs (Kobayashi *et al*., 1978), cats (Dempsey *et al*., 1971), rabbits (Freer *et al*., 1976; Ishihata & Endoh, 1995), hamsters (Hirakata *et al*., 1990), cows (Rogers, 1984) and man (Lenz *et al*., 1995), whereas no such effects could be observed in guinea-pig hearts (Baker & Singer, 1988). In the rat, the contractile response to angiotensin II remains to be a subject of controversy since opposing results have been obtained by different investigators (Neyses & Vetter, 1990; Kobayashi *et al*., 1995). Our data indicate a weak positive inotropic response to high doses of angiotensin II in atrial myocardium, whereas no positive inotropic effect of angiotensin II could be shown in the left ventricle. Therefore, it may be speculated that in rats AT_1 receptors are coupled to signalling cascades involved in growth and differentiation rather than to signalling pathways engaged in contraction of cardiomyocytes.

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