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ORIGINAL ARTICLE

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Overexpression of the human angiotensin II type 1 receptor in the rat heart augments load induced cardiac hypertrophy

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Abstract Angiotensin II is known to stimulate cardiac hypertrophy and contractility. Most angiotensin II effects are mediated via membrane bound AT_1 receptors. However, the role of myocardial AT_1 receptors in cardiac hypertrophy and contractility is still rarely defined. To address the hypothesis that increased myocardial AT_1 receptor density causes cardiac hypertrophy apart from high blood pressure we developed a transgenic rat model which expresses the human AT_1 receptor under the control of the α-myosin heavy-chain promoter specifically in the myocardium. Expression was identified and quantified by northern blot analysis and radioligand binding assays, demonstrating overexpression of angiotensin II receptors in the transgenic rats up to 46 times the amount seen in nontransgenic rats. Coupling of the human AT_1 receptor to rat G proteins and signal transduction cascade was verified by sensitivity to GTP-γ-S and in-

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creased sensitivity of intracellular Ca^{2+} $[Ca^{2+}]_i$ to angiotensin II in fluo-3 loaded transgenic cardiomyocytes. Transgenic rats exhibited normal cardiac growth and function under baseline conditions. Pronounced hypertrophic growth and contractile responses to angiotensin II, however, were noted in transgenic rats challenged by volume and pressure overload. In summary, we generated a new transgenic rat model that exhibits an upregulated myocardial AT_1 receptor density and demonstrates augmented cardiac hypertrophy and contractile response to angiotensin II after volume and pressure overload, but not under baseline conditions.

Keywords Transgenic rats · Angiotensin II receptors · Heart Hypertrophy

Abbreviations *Ang II:* Angiotensin II · *GAPDH:* Glyceraldehyde-3-phosphate dehydrogenase · GTP-γ-S*:* Guanine triphosphate analogous · *αMHC:* α-Myosin heavy chain · *RAS:* Renin-angiotensin system · *SD:* Han:Sprague-Dawley · *TGR:* Transgenic rat TGR (αMHC-h AT_1)594-17

Introduction

Angiotensin II (Ang II), a key regulator of cardiovascular homeostasis, is thought to participate in cardiac hypertrophy and remodeling since inhibitors of the renin-angiotensin system (RAS) are highly cardioprotective [1, 2]. Recent studies have suggested that Ang II stimulates cardiac growth not only via increased blood pressure but also through direct effects on cardiomyocytes [2, 3, 4]. In cultured rat cardiomyocytes it has been shown that Ang II causes many characteristics of cardiac hypertrophy, such as the increased expression of the immediate early gene c-*fos* [5], the activation of the fetal gene *ANP* [6], the organization of actin fibers into myofibrils [7, 8], and increased protein synthesis [3, 9]. In addition, the cardiac expression of all members of the RAS has been demonstrated. Furthermore, an increased local RAS activity and Ang II production in the hypertrophied/ischemic heart and in stretched cardiomyocytes, respectively, have been shown [2, 4, 10, 11]. Thus, Ang II seems to control cardiac growth and function at the autocrine/paracrine level, independently of systemic actions. However, neither the mechanisms nor the pathways of these actions are clearly defined. Data obtained from in vitro studies on rat cardiomyocytes are not conclusive. Numerous investigators have reported a modest (10–25% increase in protein/DNA ratio) or no effect of Ang II on cardiomyocyte growth in vitro [6, 9, 12]. Various studies suggest an increased delivery of other growth factors by nonmyocytes acting as a go-between for the Ang II induced cardiomyocyte growth [12, 13]. Ang II mediates its effects by interaction with two distinct types of cell surface receptors. Although these receptors are localized in the heart in roughly equivalent amounts [14], Ang II exerts most of its known effects through the AT_1 receptors, which are localized on both rat cardiomyocytes and nonmyocytes [6, 9]. Although the AT_1 receptor is abundantly expressed in the neonatal rat heart, the level of expression is quite low in the adult heart. Since AT_1 receptor expression is increased again in remodeling hearts such as cardiac hypertrophy and infarction [15, 16, 17], it was speculated that an upregulated myocardial AT_1 receptor density causes cardiac hypertrophy.

In order to address the hypothesis that increased AT_1 receptor density on cardiomyocytes is sufficient to initiate hypertrophic growth and modify contractility, we created a transgenic rat model which overexpresses the human AT_1 receptor under the control of the α-myosin heavy chain promoter (αMHC) selectively in cardiomyocytes.

Methods and materials

Generation of transgenic rats

A 1.03-kb fragment of the rat αMHC promoter [18] and a 2.19-kb fragment of the human AT_1 receptor cDNA, containing the entire coding region as well as 260 bp of the 5′ and 854 bp of the 3′ flanking regions, followed by the intron and polyadenylation signal of the SV40 T antigen were subcloned into pBSKII(+) plasmid. The resultant approx. 4.7-kb transgene was released from the plasmid by *Xba*I/*Kpn*I digestion and used to produce five transgenic founders [TGR (αMHC-h AT_1)] by pronuclear injection into fertilized oocytes derived from Han:Sprague-Dawley rats (SD) as previously described [19]. Presence of the transgene in genomic DNA from tail biopsy specimens was verified by Southern blotting using a ^{32}P-labeled 1.5-kb *Hin*dIII fragment of the human AT_1 receptor cDNA as a probe specific to the transgene.

Analysis of RNA expression

For RNA preparation from tissue the animals were killed by decapitation under ether anesthesia, and the organs were isolated, weighed, snap-frozen in liquid nitrogen, and kept at –80°C. Total RNA was extracted by the lithium chloride method modified according to Auffray and Rougeon [20]. Then 20 µg RNA was size-fractionated on a 1% formaldehyde-agarose gel and transferred to nylon membranes (Hybond N; Amersham, Braunschweig, Germany). Northern blot hybridization was performed according to standard procedures using a random-primer ^{32}P-labeled 1.5-kb *Hin*dIII DNA probe corresponding to the 5′ end of the human AT_1 receptor cDNA. This probe does not cross-hybridize with the wild-type rat AT_1 receptor mRNA, as we demonstrated in studies using RNA from nontransgenic rat heart and kidney (Fig. 1A). Rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels were used as loading standard.

Membrane preparation and binding assay

Membrane preparations from hearts and receptor binding assays were performed as described elsewhere [17]. In brief, membranes (50 µg protein) were incubated with ^{125}I-labeled [Sar^1-Ile^8]-Ang II (2200 Ci/mmol, purchased from DuPont, NEN; 0.09–1.8 nmol/l) for saturation experiments and with ^{125}I-Ang II (1 nmol/l) for competition experiments in a total assay volume of 150 µl for 90 min at 22°C. Competition experiments were carried out in the presence of PD123319 (a gift from Park Davis, Ann Arbor, Mich., USA), CV11974 (Alseda Pharmaceutical Industries Osaka, Japan) or guanosine 5′-O-(thiotriphosphate) analogous (GTP-γ-S) (at 10^{-9}–10^{-5} mol/l). All assays were run in duplicate. The measurements of ligand binding affinity (K_D) and density (B_{max}) were obtained from the saturation isotherms and the Scatchard plots generated using the InPlot program (GraphPAD Software for Science, San Diego, Calif., USA).

Intracellular Ca^{2+} imaging using confocal microscopy in single cardiomyocytes

$[Ca^{2+}]_i$ concentrations were measured in single neonatal cardiomyocytes (isolated according to Wallukat et al. [21]) by the fluo-3 technique and confocal microscopy. Myocytes attached to glass coverslips at a density of $2.40\times10^5/cm^2$ were incubated in a 5 µmol/l fluo-3 AM containing hydroxyethylpiperazine ethane sulfonic acid solution at 37°C in the dark for 20 min. For each rat strain, TGR and SD, four separate cultures of primary cardiomyocytes were analyzed. Measurements were performed on a Nikon Diaphot inverted microscope with an MRC 600 confocal imaging system (Bio-Rad) using an argon laser. Cells were exposed to increasing concentrations of Ang II (1 pmol/l–100 µmol/l).

Animal experiments

All experiments were performed using homozygous transgenic rats of the line TGR (αMHC-hAT_1)594-17 and age-matched wild-type SD rats. The rats were kept on a 12-h light-dark cycle with 55% humidity at an ambient temperature of 23±2°C and given food and tap water ad libitum. The studies were approved by the institutional animal care review committee and performed according to the laws on animal care in the state of Berlin, Germany.

Blood pressure was measured in male rats aged 8 weeks with a telemetric pressure transducer (Data Science) implanted in the abdominal aorta of the rats as previously described [22]. The system monitors blood pressure and heart rate at 10-min intervals, while the rats are moving freely. Telemetric recording was analyzed over a 8-day period to determine basal levels. Then over a 13-day period Ang II was infused subcutaneously (230 ng/kg per minute) using osmotic minipumps (Alzet 2001), which were connected to the jugular vein by a PE-50 catheter. During and after the treatment telemetric recording was analyzed. Then the rats were killed and heart/body weights assessed.

In a separate experiment central venous and mean arterial pressures were measured under chloral hydrate anesthesia in 11-week-old male rats by cannulating the right jugular vein and the right carotid artery using a PE-50 tubing catheter. The catheters were advanced into the right and left ventricles, respectively, for measurement of ventricular pressures. All pressures were recorded with a Statham transducer (P23XL) and a Gould AMP 4600 amplifier and analyzed by a Gould DIF 4600 differentiator.

The aortocaval shunt was induced in 7-week-old male rats by an 1.8-mm disposable needle using a modified method of Garcia and Diebold [23] as described previously [24]. Hemodynamic measurements were assessed 4 weeks after the surgery as described above and heart/body weights determined.

Aortic ligation was performed in male rats aged 7 weeks as described previously [25] using a blunted 0.65-mm needle for constricting the abdominal aorta to the diameter of the needle. Heart and body weights were obtained 8 weeks after the surgery.

Langendorff studies were performed in hypertrophied hearts from male rats 8 weeks after aortic ligation and in nonhypertrophied hearts from sham-operated rats. The heart was rapidly excised and arrested in ice-cold 0.9% NaCl. The aorta was immediately retrogradely perfused with a modified Tyrode solution and equilibrated with 95% O_2 and 5% CO_2. Perfusion pressure was maintained at 60 mmHg and the temperature kept between 38.0°C and 38.5°C. After equilibration for 15 min baseline parameters were measured. Thereafter Ang II was given in three doses (10^{-9}, $10^{-8.5}$, 10^{-8} mol/l) and the effect on coronary flow and d*P*/d*t* assessed.

Statistics

Values are expressed as mean and SEM. Statistical significance was determined by paired and unpaired *t* test as appropriate or one- and two-way analysis of variance with Bonferroni's correction to compensate for multiple testing. A *P* level less than 0.05 was considered statistically significant.

Results

Generation of transgenic rats overexpressing the human AT_1 receptor in the myocardium

Five transgenic rat lines were generated. Three lines expressed the transgenic AT_1 receptor in the myocardium. No evidence of developmental defects or obvious morphological changes were observed in atrial and ventricular myocardium in these lines. One transgenic line TGR (αMHC-hAT_1)594-17 was used for further studies as it exhibited the highest level of transgene expression and Ang II receptor binding in the heart. Northern blot analysis revealed transgene expression, driven by the αMHC promoter, in atria and ventricles corresponding to the endogenous αMHC, which is the predominant heavy chain

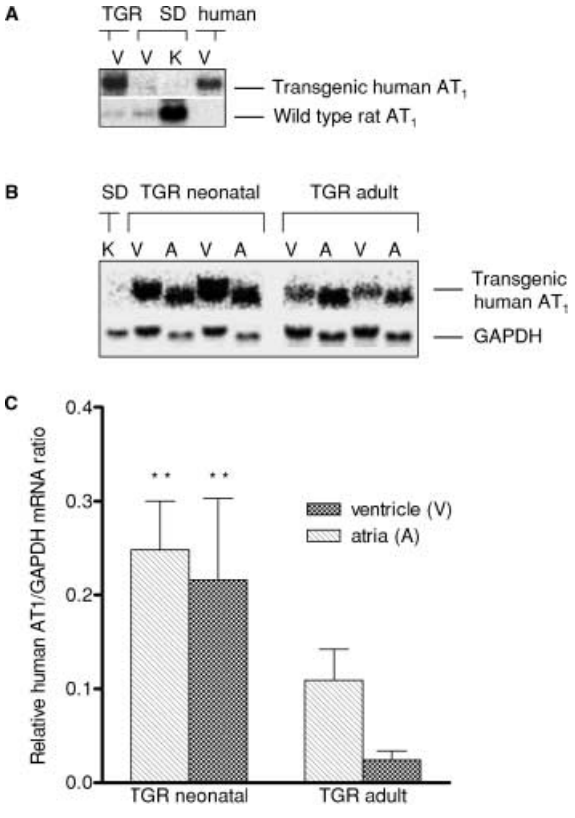

Fig. 1A–C Northern blot analysis of transgene expression. **A** Specificity of the human AT_1 receptor probe. RNA (20 µg/lane) from ventricles (*V*) of transgenic (*TGR*) and Sprague Dawley wild-type (*SD*) rats and of humans as well as from SD rat kidneys (*K*) were hybridized with a 1.5-kb human AT_1 receptor cDNA probe and subsequently with a probe corresponding to the wild-type rat AT_1 receptor. **B** Typical autoradiographs of human AT_1 receptor mRNA and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA (20 µg/lane) in atria (*A*) and ventricles (*V*) from neonatal and adult (12-week-old) TGR. Kidney (*K*) from SD rats were used as a negative control for the transgenic receptor, since it exhibits high level of wild-type rat AT_1 receptor expression in contrast to heart tissue. **C** Bar graphs of human AT_1 receptor mRNA values normalized to GAPDH expression in atria and ventricles of neonatal and adult TGR. *Each bar* (*n*=5) Mean and SEM. ***P*<0.001 vs. TGR adult

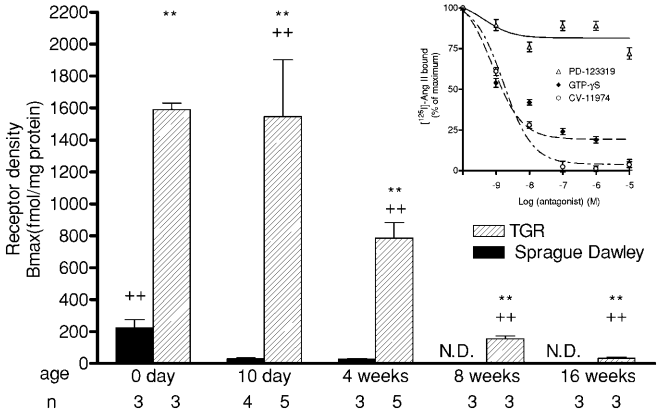

Fig. 2 Binding studies on heart membranes. Age-related changes in ^{125}I-[Sar^1,Ile^8]-angiotensin II binding (B_{max}) in heart membrane fractions from transgenic (*TGR*) and Sprague Dawley rats. Data shown were obtained from Scatchard analyses and represent mean and SEM for duplicate determination for each heart. *Insert* Competitive binding of ^{125}I-angiotensin II to heart membranes of transgenic rats (4 weeks old). ^{125}I-Angiotensin II (1 nmol/l) was incubated with heart membrane preparations in the absence and presence of the designated concentrations of AT_1 receptor specific antagonist CV11974, of the AT_2 receptor specific antagonist PD123319 and of the nonhydrolyzable GTP analog GTP-γ-S. Results are expressed as the percentage of specific ^{125}I-angiotensin II binding and values are given as mean and SEM of duplicate determinations for each of three hearts. *N.D.* Not detected; $^{**}P<0.001$ vs. wild-type rats; $^{++}P<0.001$ vs. neighboring age group

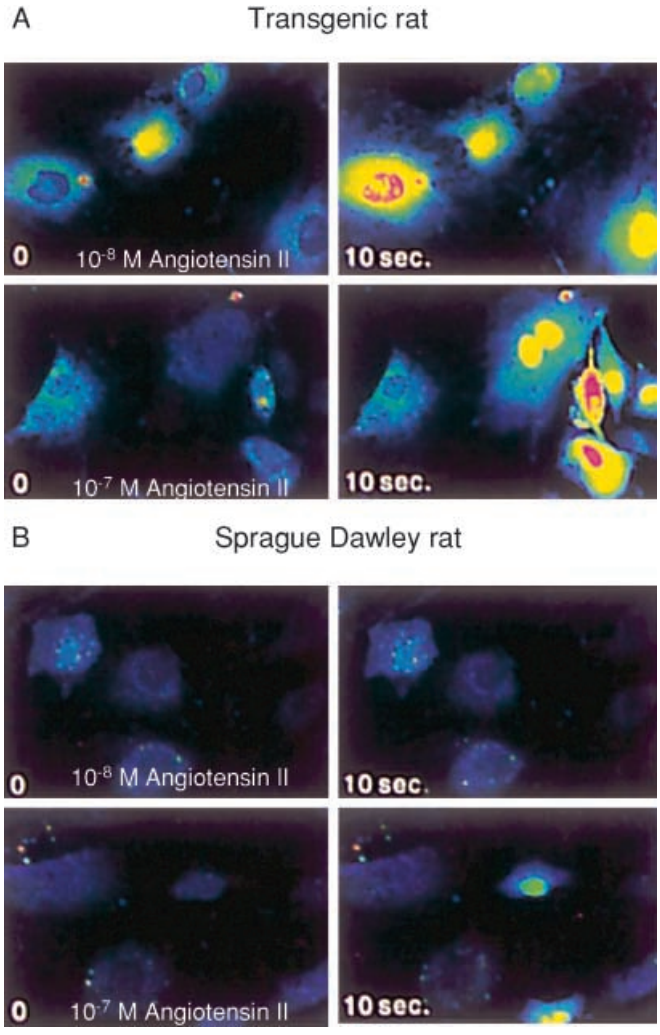

Fig. 3 $[Ca^{2+}]_i$ response to angiotensin II in single cardiomyocytes. Neonatal fluo-3 loaded cardiomyocytes from transgenic (**A**) and Sprague Dawley rats (**B**) were stimulated with angiotensin II. Figures show $[Ca^{2+}]_i$ before and 10 s after exposure to 10^{-8} or 10^{-7} mol/l angiotensin II. Measurements were performed with a MRC 600 confocal imaging system (Bio-Rad) using an argon laser. For each rat strain four separate experiments were performed

isoform in the adult atria and ventricles (Fig. 1B). Reverse transcriptase polymerase chain reaction analysis using RNA from both isolated cardiomyocytes and nonmyocytes demonstrated that the transgene was expressed exclusively in cardiomyocytes, not in nonmyocytes (data not shown). The human AT_1 receptor mRNA levels were significantly higher in the neonatal atria and ventricles than in adult hearts (Fig. 1B, C). Receptor binding assays were performed on membranes from TGR and SD rat hearts aged 0 and 10 days and 4, 8, and 16 weeks to quantify the Ang II receptor protein level throughout the development. Cardiac Ang II receptor density in SD rats decreased shortly after birth and was undetectable by binding studies by the age of 8 weeks. Ang II receptors in the neonatal transgenic hearts were overexpressed up to 46 times the amount seen in wild-type SD rat hearts. Although Ang II receptor densities in TGR declined with age, they were considerable and significantly higher throughout the development than those of age-matched SD rats (Fig. 2).

Competitive inhibition experiments using ^{125}I-Ang II as ligand revealed that exclusively AT_1 receptor protein was overexpressed, since the ligand was completely displaced by CV11974, a AT_1 receptor specific antagonist, but not by PD123319, a AT_2 receptor specific antagonist (Fig. 2, insert).

Coupling of the human AT_1 receptor to rat intracellular signaling cascades

The transgenic receptors interacted with rat G proteins since GTP-γ-S, the nonhydrolyzable GTP analog, displaced bound ^{125}I-Ang II from transgenic heart membranes (Fig. 2, insert). To examine whether the transgenic AT_1 receptor increases the $[Ca^{2+}]_i$ response to Ang II we determined the $[Ca^{2+}]_i$ change upon Ang II stimulation in single neonatal cardiomyocytes by fluo-3 AM fluorescence using confocal microscopy. Ang II induced a rapid and sustained increase in $[Ca^{2+}]_i$ in transgenic cardiomyocytes in the presence of 10^{-12} mol/l Ang II. Maximum response was observed in the presence of 10^{-8} mol/l Ang II (Fig. 3). In contrast, much higher Ang II concentrations (10^{-4}) were necessary to induce a moderate $[Ca^{2+}]_i$ response in wild-type cardiomyocytes from SD rats.

Table 1. Cardiac performance at baseline and four weeks after aortocaval shunting

Parameters	Baseline		Aortocaval shunt	
	SD (n=10)	TGR (n=9)	SD (n=10)	TGR (n=11)
Mean arterial pressure, mm Hg	105.2±2.9	104.3±6.0	78.4±3.4	79.6±5.2
Left ventricular systolic pressure, mm Hg	134.0±5.6	134.0±8.7	108.8±3.3	104.9±5.5
Left ventricular end-diastolic pressure, mm Hg	1.8±1.4	3.8±3.2	10.2±1.2	10.3±1.3
Central venous pressure, mmHg	3.2±1.6	4.1±3.0	8.2±0.6	6.8±1.0
Right ventricular pressure, mm Hg	37.6±5.9	40.6±2.3	42.8±1.6	40.8±1.5
Right ventricular end-diastolic pressure, mm Hg	2.0±1.13	2.6±1.85	9.3±1.1	6.9±1.3
Left ventricular dP/dt max (mm Hg/s)	5764±897	5427±676	4300±208	4031±321
Right ventricular dP/dt max (mm Hg/s)	1708±310	1700±269	1620±105	1565±109

Values are expressed as mean and SEM.
Data were measured in 11-week-old male rats under chloral hydrate anesthesia.
dP/dt indicates first derivative of the left and right ventricular pressure, respectively.

Hemodynamic and cardiac function

Homozygous TGR (*n*=10) were indistinguishable from SD rats (*n*=8) in terms of systolic blood pressure (SD, 131.0±3.5; TGR, 127.1±4.3 mmHg), diastolic blood pressure (SD, 87.9±4.1; TGR, 85.5±3.4 mmHg), and heart rate (SD, 359.6±13.1; TGR, 365±13.2 beats/min) as telemetric studies in conscious rats revealed. Furthermore, blood pressure, left and right ventricular functions measured in anesthetized rats (Table 1), and heart/body weights (Fig. 4B) were similar between wild-type and transgenic rats. Histological analysis did not reveal any evidence for myocyte necrosis, hypertrophy, or fibrosis (data not shown).

In vivo response to Ang II

Chronic infusion of 230 ng/kg per minute Ang II for 13 days caused a significant increase in systolic blood pressure. The increase in relative heart weights was minor but significant. These changes were similar in both TGR and SD rats (Fig. 4B).

Volume or pressure overloading

Pressure overloading by interrenal aortic banding was carried out for 8 weeks since our previous experiments indicated that after 8 weeks a stable phase of hypertrophy is reached [26]. Volume loading by aortocaval shunting, however, is an immediate more severe hemodynamic burden [24]. Therefore to avoid an excessive period of this severe loading we finished the volume overload experiments after 4 weeks, before the rats would die from heart failure. Both stimuli induced a significant hypertrophic response in TGR and SD rats as indicated by heart/body weight ratios (Fig. 4B). These hypertrophic responses were significantly more pronounced in TGR than in SD rats. As shown in Fig. 4A, the high baseline Ang II receptor density in TGR tended to be further increased by 40–140% during hypertrophic

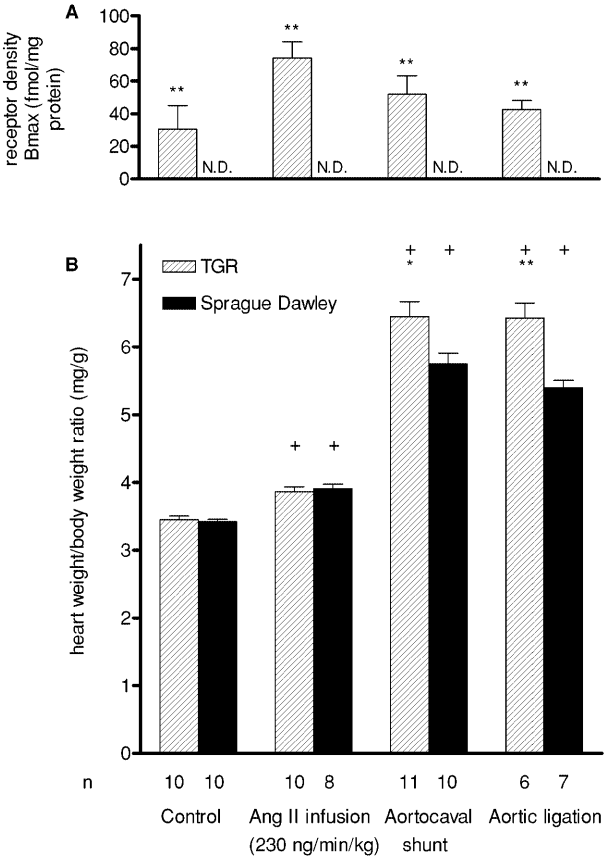

Fig. 4 Effects of angiotensin II infusion, aortocaval shunting, and chronic aortic ligation on cardiac angiotensin II receptor densities (**A**) and heart/body weight ratios (**B**) in transgenic (*TGR*) and in Sprague Dawley rats. In 9-week-old rats angiotensin II was infused for 13 -days using osmotic minipumps. Aortocaval shunting and aortic ligation were performed in 7-week-old rats for 4 weeks or 8 weeks. Age-matched sham-operated rats were used as controls. Binding studies on heart membrane preparations obtained from these rats demonstrate a significantly higher angiotensin II receptor density in TGR rats than in Sprague Dawley rats. Data represent mean and SEM. *N.D.* not detected; $^{**}P<0.001$, $^{*}P<0.05$ vs. wild-type rats; $^{+}P<0.001$ vs. controls

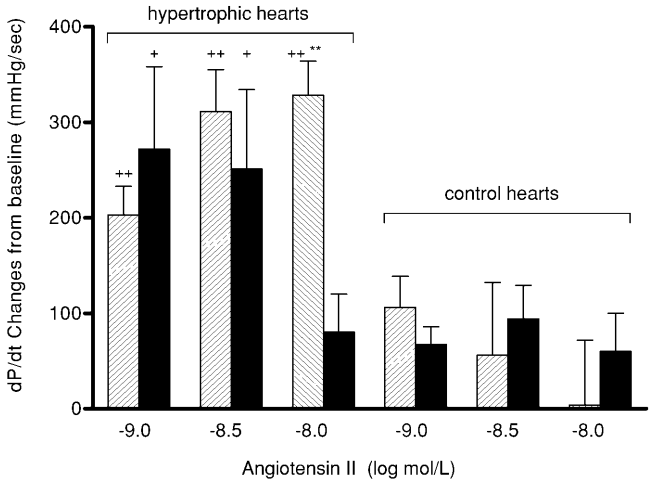

Fig. 5 Effect of angiotensin II on first derivative of peak developed ventricular pressure (*dP/dt max*). Graphs show relative-to-baseline changes in systolic d*P*/d*t* in response to indicated concentrations of angiotensin II measured in retrogradely perfused hearts in a Langendorff mode; Hypertrophic (induced by aortic ligation) and nonhypertrophic control (sham-operated) hearts were obtained from transgenic (*shaded bars*; hypertrophic, *n*=5; control, *n*=6) and Sprague Dawley rats (*black bars*; hypertrophic, *n*=7; control, *n*=6). Data are expressed as mean and SEM. $^{**}P<0.001$ vs. Sprague Dawley rats; $^{++}P<0.001$, $^{+}P<0.05$ vs. baseline

response, while Ang II binding was not detected in either the control or hypertrophied SD hearts.

At the endpoints of the experiments we characterized cardiac function in vivo (aortocaval shunt model) and in isolated retrogradely perfused hearts (aortic banding) to address different questions. The hemodynamics 4 weeks after aortocaval shunting in vivo did not differ significantly between TGR and SD rats (Table 1) indicating similar systemic and functional cardiac responses to volume overload. Therefore we determined in vitro cardiac function after 8 weeks of pressure overload since the effect of circulating Ang II should be avoided. Cardiac function including coronary flow and d*P*/d*t* were similar in TGR and SD rats (data not shown).

Langendorff experiment

We examined cardiac function using isolated retrogradely perfused hearts in the presence or absence of Ang II. Hearts were obtained from TGR and SD rats 8 weeks after aortic ligation as well as from the respective sham-operated rats. Under baseline conditions no difference was found in any parameter between both strains, including coronary flow and d*P*/d*t*. Perfusion with Ang II at 10^{-9} mol/l increased systolic and diastolic d*P*/d*t* in the hypertrophied hearts of both strains. These increases were sustained in TGR hearts while they were transient in SD hearts. In contrast, no response in d*P*/d*t* to Ang II was observed in hearts from either sham-operated TGR or SD rats (Fig. 5). The effects of Ang II on the coronary flow were similar in TGR and SD (data not shown).

Discussion

This study characterizes for the first time a new transgenic rat model which overexpresses the human AT_1 receptor under the control of the αMHC promoter in cardiomyocytes. The transgenic receptor is expressed abundantly in the atria and ventricles and coupled to rat intracellular signaling pathways as evidenced by the receptor interaction with G proteins and enhanced $[Ca^{2+}]_i$ sensitivity in response to Ang II. Since AT_1 receptors are known to be linked to $[Ca^{2+}]_i$ signaling pathways, we conclude that the human AT_1 receptor is functionally active in transgenic rats. We noted pronounced hypertrophied growth and contractile response to Ang II in TGR challenged by volume and pressure overload, while under baseline conditions TGR exhibited normal cardiac growth, morphology and function. The importance of myocardial AT_1 receptors for the development of cardiac hypertrophy is still unclear. In different models of cardiac hypertrophy angiotensin-converting enzyme inhibitors and AT_1 receptor antagonists have been shown to be highly protective [1, 4]. However, those studies do not confirm whether the protective effect is due to blocking the systemic or the local cardiac actions of Ang II. In vitro studies on isolated cardiomyocytes provide controversial results regarding growth effects of Ang II. Recent studies using pure cultures of cardiomyocytes have indicated that Ang II alone may not be sufficient to induce a growth response in cardiomyocytes, and that the observed growth effect might be mediated via Ang II induced release of paracrine factors from nonmyocytes [12]. These studies are supported by our findings showing that selective overexpression of the human AT_1 receptor on rat cardiomyocytes is not sufficient to induce a hypertrophic response.

However, our data are partly at odds with the findings of two other studies in different transgenic mouse models with αMHC promoter regulated cardiac overexpression of the murine or the human AT_1 receptor. These models developed a distinct cardiac phenotype. When the murine AT_1 receptor was overexpressed, the mice died shortly after birth as a consequence of massive atrial enlargement due to myocyte hyperplasia and distinctly abnormal excitation-contraction coupling. A clear ventricular phenotype was not present [27]. The overexpression of the human AT_1 receptor in the mouse heart, however, did not mediate such a dramatic phenotype despite the transgene being expressed at considerably higher levels, but caused ventricular hypertrophy in adult mice [28]. In our TGR the human AT_1 receptor is highly expressed in the atria and ventricles even in neonatal rats, but we did not detect any sign of hyperplasia or hypertrophy in either the atria or ventricles until further stimuli were added. The transgenic AT_1 receptor in our TGR, as well as in the other two mouse models, is regulated by the αMHC promoter, the activity of which is restricted before birth to immature atrial cardiomyocytes and postnatal to atrial and ventricular cardiomyocytes, which lost their hyperplastic capacity. Altogether, data from these

transgenic studies suggest that the AT_1 receptor overexpression causes hyperplasia in immature and hypertrophy in terminal differentiated cardiomyocytes. Although being controlled by the same promoter, a different prenatal onset and level of transgene expression could be one reason that atrial hyperplasia was detected only in one of the three transgenic models. Another reason could be different signaling of mouse versus the human AT_1 receptors. However, there is a high degree of homology between the human and mouse AT_1a receptor, not only within the coding region (sharing 95% protein sequence identity) but also in the 5′- and particular 3′-nontranslated region. When stable expressed in Chinese hamster ovary cells, both receptors were shown to interact with the same three types of G proteins – Gq, Gi, Go [29]. Therefore it is unlikely that the human and mouse AT_1 receptor signaling would differ in the mouse heart.

Recent studies indicate an importance of the 3′-untranslated region for the AT_1 receptor mRNA stability and for coupling to intracellular signaling [29]. It has been shown that the normally unstable AT_1 receptor mRNA becomes highly stable by removing its 3′-untranslated region, and that the coupling to intracellular signaling differs between AT_1 receptors with and without the 3′-untranslated region. We have no information on whether the mouse AT_1 receptor transgene used in the mouse model [27] contained the 3′-untranslated region. For our study we used the full-length human AT_1 receptor cDNA including the 854 bp of the 3′-untranslated region. In contrast, the human AT_1 receptor transgenic mice [28] harbored only the coding region and a very short sequence of the 3′-untranslated region. Perhaps this explains the high human AT_1 receptor expression throughout life in transgenic mice, while in our TGR the receptor levels decreased with age. In addition, it is conceivable that long-term high-level Ang II stimulation is necessary for induction and progression of hypertrophic changes. Finally, it should be mentioned that all three transgenic AT_1 receptors were acting on a different genetic background. Probably strain and species differences in the activity of the local RAS and the expression level of different types of G proteins or mRNA binding proteins to the 3′-untranslated region could influence the receptor signaling in quality and quantity. Since we used the full-length cDNA of the AT_1 receptor, our TGR represents a good model for studying the function of the intact, not mutated human AT_1 receptor.

We cannot exclude that effects of increased AT_1 receptors in our model are masked by compensatory mechanisms. Since it has been reported that the AT_2 receptor tempers AT_1 receptor mediated cell growth at the level of signal transduction in several cell types including cardiomyocytes [6, 30], we showed that AT_2 receptors were expressed at almost the same levels in both TGR and SD rat hearts. Hence AT_1 receptor actions were not masked by endogenous AT_2 receptors in our TGR.

A compensatory downregulation of cardiac Ang II levels might minimize transgene effects in our rats. Therefore we stressed the transgenic and control rats for several weeks by volume and pressure overload using aortocaval shunting and aortic ligation, respectively, which activate the local RAS and induce hypertrophied growth [11, 25]. Our binding studies indicated that in transgenic hearts the high baseline Ang II receptor density tended to further increase during hypertrophy. Such an increase was not detectable in hypertrophied SD hearts. However, increased mRNA levels of rat AT_1 receptor in both TGR and SD hearts were demonstrated. In addition, the human AT_1 receptor level was increased (aortic ligation) or not changed (aortocaval shunt) in transgenic hearts (data not shown). We believe that the type of binding studies which we performed was not sensitive enough to detect a modest change in the low level of Ang II binding in SD hearts. Under both pathophysiological conditions the transgenic rats revealed a significantly pronounced cardiac hypertrophy, as assessed by heart/body weight ratios. However, infusion of pressure doses of Ang II for 13 days, sufficient to evoke a significant increase in relative heart weights, did not cause any additional growth effect in TGR hearts when compared with wild-type SD rats.

These data suggest that increased Ang II actions alone may not be sufficient to initiate hypertrophic growth. Therefore we stimulated isolated nonhypertrophied and hypertrophied hearts from both transgenic and nontransgenic rats in a Langendorff experiment with different doses of Ang II. Ang II elicited positive inotropic effects in hypertrophied hearts of both TGR and SD rats, but not in normal hearts. The expected tachyphylaxis of Ang II was clearly blunted in transgenic hearts, which demonstrates the functionality of the transgenic Ang II receptors. The different effects of overexpressed transgenic AT_1 receptors in normal and hypertrophied hearts in our TGR led us to speculate that the cardiac AT_1 receptors activate distinct signaling pathways or exhibit a different coupling efficiency under baseline and pathophysiological conditions. Reexpression of fetal genes in the hypertrophied hearts or activation of other growth factors such as endothelin-1 could supply cofactors which are necessary to link the AT_1 receptor signaling to the initiation of reactive hypertrophy under pathophysiological conditions [5, 6, 13, 15]. Therefore our data may underscore the contribution of transgenic human AT_1 receptors on rat cardiomyocytes to hypertrophied growth induced by other factors.

In summary, we developed a transgenic rat model with myocardial overexpression of the human AT_1 receptor to study in vivo the consequences of increased AT_1 receptor density in the myocardium apart from other Ang II effects. We demonstrated that AT_1 receptors on cardiomyocytes participate in cardiac hypertrophy caused by pressure or volume overload, but are not linked to cell growth in physiological conditions. Furthermore, Ang II elicited positive inotropic effects in hypertrophied but not in nonhypertrophied hearts. These findings indicate that myocardial AT_1 receptors in the rat system contribute to hypertrophic growth induced by other factors but may not cause directly cardiomyocyte

hypertrophy. Taken together, our data suggest a distinct role of myocardial AT_1 receptors in normal and hypertrophied hearts, possibly due to a different coupling of the receptors to the intracellular signaling pathways.

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