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Overexpression of angiotensin AT_1 receptor transgene in the mouse myocardium produces a lethal phenotype associated with myocyte hyperplasia and heart block

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ABSTRACT Previous studies have suggested that angiotensin II (Ang II) modulates cardiac contractility, rhythm, metabolism, and structure. However, it is unclear whether the cardiac effects are due to direct actions of Ang II on the myocardium or if they are due to secondary effects mediated through the hemodynamic actions of Ang II. In this study, we used the α-myosin heavy chain (αMHC) promoter to generate transgenic mice overexpressing angiotensin II type 1 (AT_{1a}) receptor selectively in cardiac myocytes. The specificity of transgene expression in the transgenic offspring was confirmed by radioligand binding studies and reverse transcription–PCR. The offspring displayed massive atrial enlargement with myocyte hyperplasia at birth, developed significant bradycardia with heart block, and died within the first weeks after birth. Thus, direct activation of AT_1 receptor signaling in cardiac myocytes *in vivo* is sufficient to induce cardiac myocyte growth and alter electrical conduction.

Angiotensin II (Ang II) plays a central role in blood pressure and water and electrolyte homeostasis. Data suggest that Ang II via its action on AT_1 receptors has a direct effect on cardiac function and structure (1), and that the activation of a local intracardiac angiotensin system may participate in cardiac pathophysiology such as myocardial hypertrophy and enlargement (2–4). These observations may explain the clinical efficiency of pharmacological inhibition of the renin–angiotensin system in preventing cardiac enlargement and in prolonging survival in patients with cardiac disease. However, the paradigm of local Ang II and cardiac remodeling is based primarily on indirect *in vitro* or *in vivo* observations (3, 5–9). It is unclear whether the growth-stimulatory effects of Ang II are directly mediated via AT_1 receptors on cardiac myocytes, are indirect effects mediated via angiotensin-induced release of paracrine factors from non-myocytes (e.g., fibroblasts), or are secondary to the systemic hemodynamic effects of Ang II. We have used the α-myosin heavy chain (αMHC) gene promoter (10) to generate transgenic mice overexpressing AT_1 receptors selectively in cardiac myocytes to address the following related questions. Can Ang II induce cardiac remodeling *in vivo* independent of its systemic hemodynamic effects and is this effect mediated by AT_1 receptors on cardiac myocytes? Can the overexpression of one component (AT_1 receptor) of the local renin–angiotensin system alter cardiac function or structure? What are the consequences of increased AT_1 receptor signaling on cardiac development and viability?

METHODS

Plasmid Construction and Generation of Transgenic Mice. A murine α-myosin heavy chain promoter–AT_{1a} receptor transgene was constructed (Fig. 1*A*). The plasmid sequence was removed by *Not*I digestion and DNA was purified by Geneclean (Bio 101). FVB/N mice were superovulated by standard procedures (11), and fertilized eggs were injected with the linearized DNA (2 ng/μl) in 5 mM Tris·HCl, 0.1 mM EDTA (pH 7.4). The injected embryos were transferred to the oviducts of pseudopregnant CD-1 mice. Genomic DNA was isolated from tail biopsies taken at 3 weeks of age or from the spleen of younger mice. Southern hybridization was carried out using the ^{32}P-labeled *Nco*I–*Hin*dIII fragment of mouse AT_{1a} receptor cDNA as a probe. For some experiments, a tyrosinase minigene (12) was coinjected with the αMHC-AT_1 DNA to allow visual identification of transgenic offspring by coat color. Germline transmission of the transgene was tested by mating male transgenic founders with superovulated wild-type FVB/N mice or by mating female transgenic founders with wild-type FVB/N males. To test whether inhibition of the renin–angiotensin system could prolong survival of transgenic mice, some female FVB/N mice that were mated with the male founder M4/7 received captopril (1 mg/ml drinking water) or losartan (30 mg/kg body weight per day i.p.) from day 12 of pregnancy until weaning of the offspring (at 3 weeks of age). The doses of captopril and losartan used in these studies are sufficient to completely block the blood pressure effects of intraarterial infusion of 5 μg/kg Ang I or Ang II, respectively, in mice (data not shown). Nontransgenic control mice had access to normal drinking water and received daily injections of 0.9% saline (0.1 ml i.p.).

Expression of the αMHC-AT_1 Transgene. Total RNA was treated with 25 units of RNase-free DNase (Stratagene) for 30 min at 37°C in 50 mM Tris·HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, and 60 units of RNasin (Promega), and were then reverse-transcribed using oligo(dT) as a primer (SuperScript preamplification system, GIBCO/BRL). Mock cDNA synthesis reactions lacking reverse transcriptase were carried out for all samples. To detect transcripts from the endogenous AT_{1a} receptor gene, the sense primer *A* (5′-CAA CCC AGA AAA GCA AAA TGG C-3′, positions −17 to +5 of the cDNA (13), was located before the open reading frame and the antisense primer *T* (5′-CGT TGA ATT CCG GGA CTC G-3′, cDNA positions 624–642) was located in the fifth transmembrane

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Abbreviations: AT_1, angiotensin II type 1 receptor; Ang II, angiotensin II; αMHC, α-myosin heavy chain; ECG, electrocardiogram; SV40, simian virus 40.

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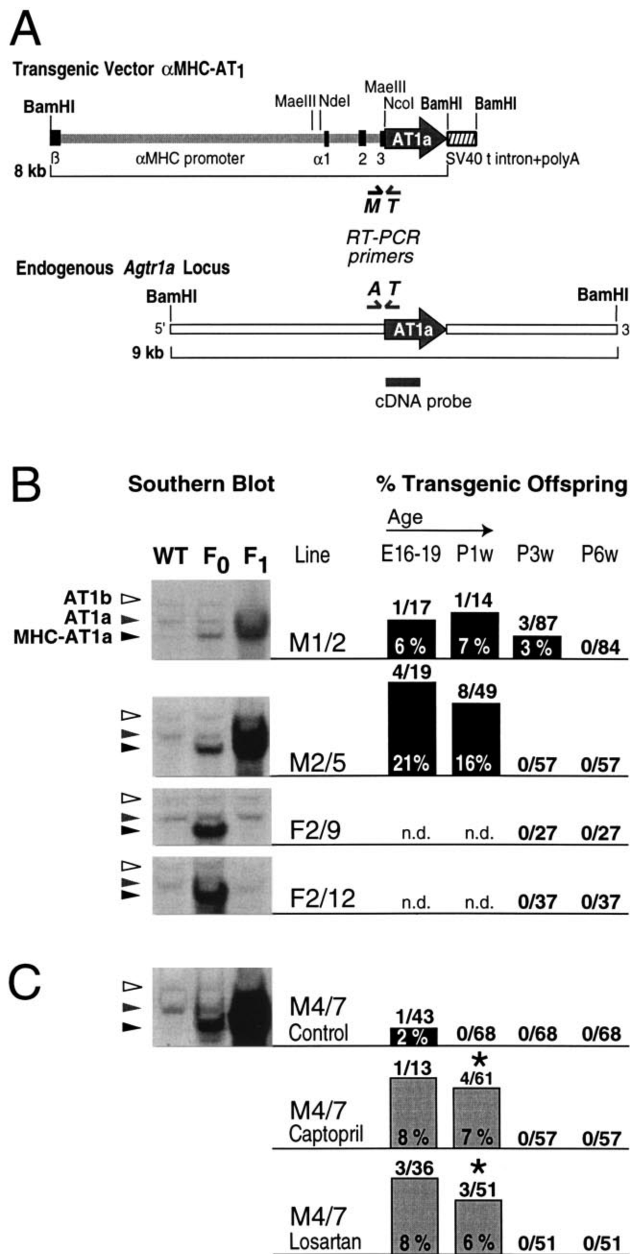

FIG. 1. Transmission rate and survival of αMHC-AT_1 transgenic mice. (*A*) Transgenic vector. The complete intergenic region between the β- and α-myosin heavy chain genes (αMHC promoter) was used as a cardiac-specific promoter to control expression of the mouse AT_{1a} angiotensin receptor. At the 3′ end of the AT_{1a} receptor cDNA, the intron and polyadenylation signal of the SV40 T antigen was added (SV40 t intron+polyA). β, α1,2,3 exons of the βMHC and αMHC locus; *Agtr1a*, angiotensin AT_1 receptor gene; *A, T, M*, primers for reverse transcriptase–PCR. (*B* and *C*) Transmission frequency of the αMHC-AT_1 transgene. Transgenic founder mice were mated with wild-type FVB/N mice, and the frequency of transmission of the transgene to their offspring was determined by Southern blot analysis using part of the coding region of the AT_{1a} receptor cDNA. The endogenous AT_{1a} gene can be detected as a 9-kb *Bam*HI fragment (shaded arrowhead), the AT_{1b} gene appears as a faint band at 10.6 kb size (open arrowhead), and the αMHC-AT_1 transgene is detectable as a 8-kb fragment (solid arrowhead). For lines F2/9 and F2/12, blots from the F1 generation represent nontransgenic offspring, as no transgenic mice were born from these lines. Fifteen micrograms of genomic DNA was loaded per lane. Transmission rates are given in absolute numbers (number of transgenic offspring/total mice screened) and in percent values for the following age groups: at embryonic days 16–19 (E16–19), at 1 week after birth (P1w), at weaning age (3 weeks, P3w), and at 6 weeks of age (P6w). (*C*) Effect of ACE inhibitor treatment or AT_1 receptor blockade on the survival of transgenic offspring of the M4/7 line. Pregnant mice were treated from day 12 after conception with captopril, losartan, or saline throughout pregnancy and during the lactation period. With both captopril and losartan, survival of the transgenic mice at 1 week of age was significantly improved as compared with untreated mice (∗, $P < 0.05$).

Table 1. Comparison of heart weight and body weight in wild-type and αMHC-AT_1 transgenic mice

Area compared	Wild type (n = 10)	αMHC-AT_1 transgenic (n = 5)
Body weight, g	3.10 ± 0.32	2.91 ± 0.26
Heart weight, mg	27.2 ± 3.1	58.1 ± 6.9*
Heart/body weight, %	0.88 ± 0.03	2.01 ± 0.07*
Ventricles weight, mg	23.2 ± 3.2	27.9 ± 4.8
Atria		
Weight, mg	3.3 ± 0.7	29.5 ± 6.9*
Wall area, mm^2	0.251 ± 0.036	0.575 ± 0.078*
Nuclei/cross-section	1,117 ± 134	2,429 ± 198*
Nuclei/mm^2 wall area	4,441 ± 532	4,219 ± 344
Cell size, μm^2	225 ± 32	237 ± 21
Cell types, %		
Myocytes	69 ± 6	68 ± 5
Fibroblasts	12 ± 3	11 ± 3
Endothelial cells	19 ± 4	21 ± 7

Transgenic offspring from line M4/7 at days 3–4 after birth show a significant increase in cardiac mass and heart/body weight ratio due to a significant increase in atrial size. Ventricular mass is not increased significantly. Morphometric analysis of atrial cross-sections reveals a significant increase in wall area and number of cell nuclei per cross-section. Data are obtained from 5 transgenic mice and 10 littermates that were derived from 4 independent litters. Mothers of these mice had received captopril (two mothers) or losartan (two mothers) during pregnancy and lactation, as described. Similar data were obtained for transgenic offspring of the line M2/5 (data not shown).
*$P < 0.01$, wild type vs. transgenic.

domain. For the transgenic transcript, the same antisense primer was used together with a sense primer (*M*) at the end of the αMHC promoter region (5′-AAG AGT TTG AGT GAC CAT GGC-3′). PCR conditions were as follows: 1 μM of each primer, 1.5 mM $MgCl_2$, 5% (vol/vol) dimethyl sulfoxide. The mixture was then amplified using the following cycle conditions: 1 min at 94°C, 1 min at 52°C, 2 min at 72°C for 35 cycles with a 10-min final extension step.

Radioligand Binding Experiments. To examine the expression of angiotensin receptors, radioligand-binding experiments were performed. From line M2/5, hearts were obtained from three wild-type and three transgenic mice 3 days after birth. The same number of cardiac specimens was obtained on postnatal days 5 and 7 from wild-type and transgenic mice of line M4/7. The mothers of offspring from line M4/7 were treated with captopril (1 mg/ml drinking water) from day 12 of pregnancy until weaning of the offspring. Cardiac specimens were homogenized in binding buffer (75 mM Tris·HCl/1 mM EDTA/125 mM $MgCl_2$, pH 7.4) in a polytron (3 × 15 sec) followed by five strokes in a Potter–Elvehjem and were subjected to centrifugation at 900 × *g*. The supernatant was centrifuged at 25,000 × *g* to obtain a membrane pellet. Saturation isotherms were obtained by incubating membranes (150 μg protein) with varying concentrations of [^{125}I]Sar-Ile-angiotensin II (2,200 Ci/mmol; 1 Ci = 37 GBq; DuPont/NEN). Specific AT_1 receptor binding was determined by the addition of 10 μM DuP753. Binding data were analyzed by nonlinear regression using INPLOT 4.0 software (GraphPad, San Diego).

Histology. Tissue samples were fixed in 4% freshly prepared paraformaldehyde in phosphate buffer, pH 7.2. After embed-

ding in paraffin, each heart was cut in 5-μm sections in 100-μm steps on a Reichert–Jung microtome. Sections were stained with hematoxylin–eosin for morphometric analysis or with Mason's trichrome to determine myocardial fibrosis. For morphometric analysis (Table 1), 4–6 sections of atria and ventricles from each heart were evaluated. The number of cardiomyocyte cell nuclei per unit area was determined as previously described (14). Photomicrographs were taken on a Zeiss Axiophot microscope, and pictures were digitized using a flatbed scanner (Apple Color Scanner) and analyzed using Adobe PHOTOSHOP 2.5.1 and NIH IMAGE 1.47 software on a Macintosh PowerPC computer.

Electrocardiogram (ECG). For these experiments, 1- to 2-day-old mice from the founder line M4/7 were used. In this case, transgenic offspring could be readily identified by their dark eye color due to insertion of the tyrosinase minigene. Mice were anesthetized with tribromoethanol (0.012 ml of 3% tribromoethanol per gram body weight by intraperitoneal injection) or methoxyflurane (1.75% by inhalation). To record the ECG, small wire electrodes were attached to the right arm and left leg and connected to a Gould amplifier and chart recorder. Heart rate was simultaneously determined by online recording using DATAFLOW software (Crystal Biotech, Hopkinton, MA).

RESULTS

A transgenic vector was constructed by combining the mouse angiotensin AT_{1a} receptor with the promoter of the α-myosin heavy chain gene (Fig. 1*A*). From the initial series of pronuclear injections, four transgenic founder mice (F_0) with varying copy numbers of the transgene were obtained (Fig. 1*B*). These founders were bred with wild-type FVB/N mice, and genomic DNA from the offspring (F_1) were screened for the presence of the transgene (Fig. 1*B*). Surprisingly, only a small percentage of offspring from the male founder M1/2 was shown to carry the transgene at weaning age (Fig. 1*B*). No transgenic offspring were obtained from the other three founder mice when screened at weaning age. However, when screened during the last week of embryonic development or 4–6 days after birth, the percentage of offspring carrying the transgene was significantly higher (Fig. 1*B*), suggesting that transgenic offspring died between birth and weaning. It is interesting to note that the transgenic founders survived much longer, between 9 and 17 months. When equal amounts of genomic DNA were compared on Southern blots, the signal intensity from the transgenic offspring was significantly higher than the corresponding signal of the founder mice (Fig. 1*B*; F_1 vs. F_0 generation). This suggests that the founder mice were mosaic due to integration of the transgenic DNA into the host genome at a later stage than the one-cell embryo.

To facilitate the identification of transgenic mice by coat color early after birth, a tyrosinase minigene (12) was used for pronuclear injection together with the αMHC-AT_1 transgenic DNA. From this series of injections, one male founder mouse was derived (Fig. 1*C*), in which transmission of the αMHC-AT_1 transgene was linked with the expression of the tyrosinase minigene (dark eye color). To explore the role of Ang II and the AT_1 receptor in the lethal phenotype, we studied the effect of pharmacological inhibition of the renin–angiotensin system on the survival of the transgenic offspring from this founder. When pregnant mice were treated with the ACE inhibitor, captopril, or the AT_1 receptor antagonist, losartan, survival of transgenic offspring was significantly prolonged (Fig. 1*C*). Neither captopril nor losartan treatment had a significant influence on the litter size or the survival of the nontransgenic mice (data not shown).

Tissue-specific overexpression of the αMHC-AT_1 transgene was determined in mice 3–7 days after birth. Transcripts for the endogenous AT_{1a} receptor could be detected in heart, lung,

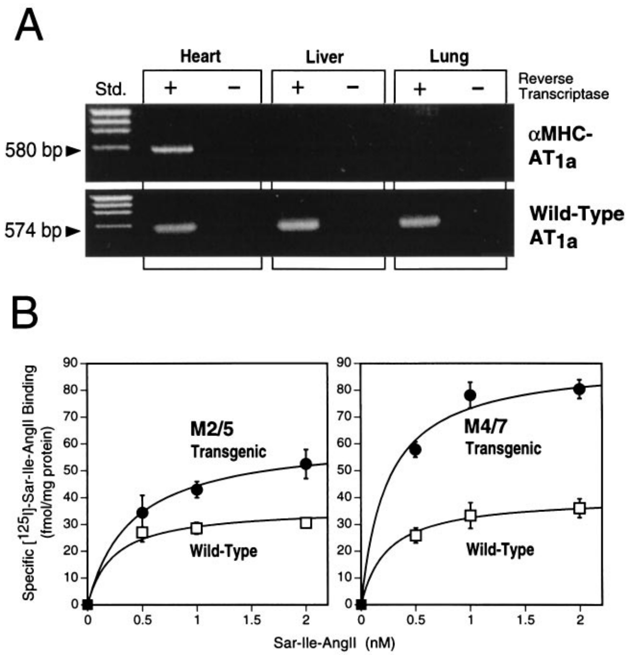

FIG. 2. Cardiac-specific expression of the αMHC-AT_1 transgene. (*A*) Detection of the expression of the αMHC-AT_1 transgene by reverse transcriptase–PCR in 3-day-old transgenic offspring of line M2/5. Messenger RNA for the endogenous AT_{1a} receptor gene could be detected in heart, lung, and liver, whereas mRNA for the αMHC-AT_1 transgene could only be detected in the heart. (*B*) AT_1 receptor saturation binding isotherms in heart membranes derived from transgenic lines M2/5 (*Left*) and M4/7 (*Right*). Hearts were obtained from transgenic mice and wild-type littermates at day 3 (line M2/5) or days 5–7 (line M4/7) after birth. Data shown represent means ± SEM for duplicate determinations of three hearts in each group. Transgenic offspring express significantly more AT_1 receptors in the heart than their nontransgenic littermates.

and liver, whereas mRNA for the αMHC-AT_1 transgene could only be detected in the heart, but not in other tissues (Fig. 2*A*). To quantitate the levels of expression of the transgene, membranes were isolated from hearts of F_1 offspring on postnatal days 3–7 and assayed for AT_1 binding sites. The expression level of AT_1 receptors in the heart of transgenic mice was increased significantly by 75% (M2/5 offspring) and 156% (M4/7 offspring) compared with nontransgenic hearts (Fig. 2*B*). Other tissues (liver, lung, kidney) did not show any difference in the amount of AT_1 receptors between wild-type and transgenic mice (data not shown).

Hearts from transgenic mice exhibited a massive enlargement of the atria. In the transgenic offspring from the high-copy founder M4/7, the atria were almost as big as the ventricles (Fig. 3*A*–*C*). Enlargement of the atria was most obvious in offspring from the high-copy lines M4/7 and M2/5, but was noted in four out of five offspring from the low-copy line M1/2, as well (data not shown). For the line M4/7, atrial enlargement was already detectable at days 16–19 of fetal development. Heart weight and heart-to-body-weight ratio of transgenic offspring from line M4/7 were increased 2.14-fold and 2.28-fold above control due to an increase in atrial mass (Table 1).

Morphometric analysis of the atria of the transgenic mice revealed that the increase in atrial size was due to hyperplasia rather than hypertrophy of atrial myocytes (Table 1). Morphology of the ventricles (Fig. 3 *D* and *E*) and ventricular myocytes did not differ between transgenic and nontransgenic hearts. In heart sections stained with Mason's trichrome, no signs of cardiac fibrosis were detectable in transgenic hearts when compared with hearts from wild-type mice (not shown). The disproportionate effect on atrial enlargement is probably

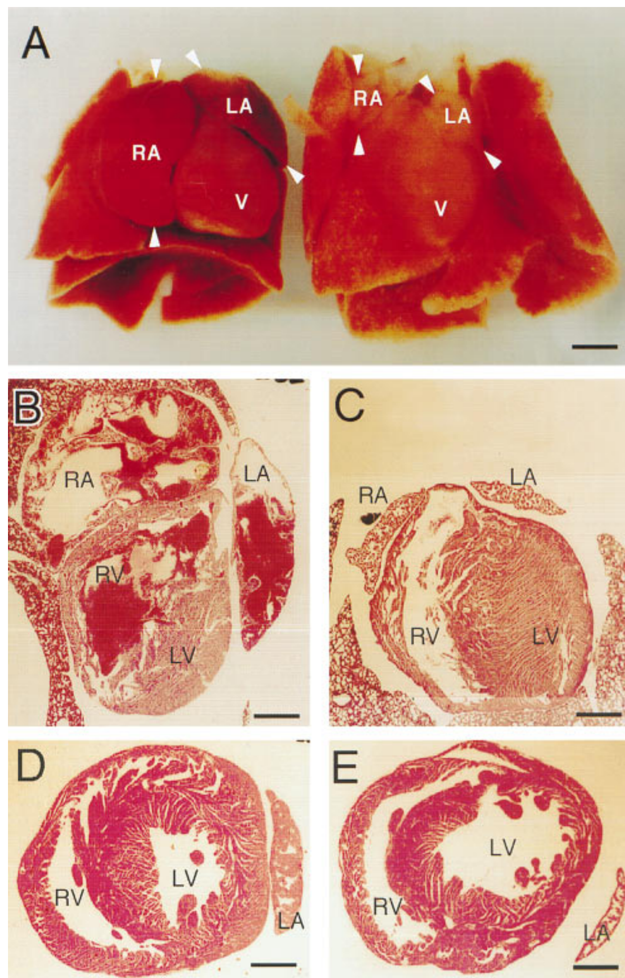

FIG. 3. Cardiac phenotype of the αMHC-AT_1 transgenic mice. (*A*) Heart and lung preparation of transgenic (*Left*) and nontransgenic (*Right*) littermates from line M4/7 at day 4 after birth. The mother of these mice was treated with captopril from day 12 of pregnancy until day 4 after delivery (1 mg/ml drinking water). Compared with the control specimen, the transgenic heart displays a massive enlargement of left and right atria. Arrowheads mark the borders of the atria. RA, right atria; LA, left atria; V, ventricle. Bar = 2 mm. (*B* and *C*) Frontal section through the same transgenic (*B*) and nontransgenic (*C*) hearts as shown in Fig. 3*A*. The atrial cavity is greatly increased in transgenic atria compared with nontransgenic atria. RA, right atria; LA, left atria; LV, left ventricle; RV, right ventricle. Bar = 1.5 mm. (*D* and *E*) Horizontal cross-sections through the ventricles of transgenic (*D*) and nontransgenic (*E*) offspring from line M4/7 do not reveal any significant morphological differences between transgenic and nontransgenic ventricles. Bar = 1.2 mm.

due to the expression of the αMHC in the atria during embryonic development of the heart (15). As the murine αMHC promoter in the ventricles becomes activated only after birth, one would predict that those transgenic mice that survive longer would develop ventricular enlargement as well.

The mechanism of the lethal effect of AT_1 receptor transgene overexpression was difficult to ascertain because most transgenic mice died *in utero* or immediately after birth. In an attempt to study this phenomenon, we recorded ECGs of newborn transgenic and nontransgenic mice under anesthesia. Transgenic mice had a significantly lower heart rate than their nontransgenic litter mates (Fig. 4). Furthermore, analysis of the ECG of transgenic mice revealed atrioventricular conduction block, as evidenced by a significant prolongation of the P–R interval [i.e., 118 ± 8 ms (mean ± SEM; n = 4) in transgenic offspring as opposed to 58 ± 6 (mean ± SEM; n = 10) in nontransgenic mice]. In addition, the widening of the

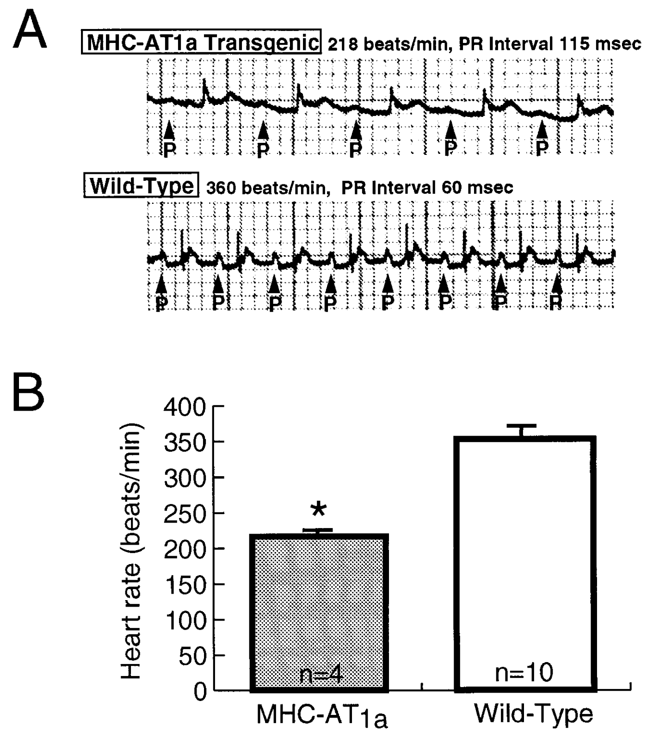

FIG. 4. Electrophysiological alterations in the αMHC-AT_1 transgenic mice. ECG of αMHC-AT_1 transgenic and nontransgenic littermate mice (offspring of line M4/7, captopril-treatment of the mothers) during the first 2 days after birth. Transgenic mice display severe bradycardia (*A* and *B*) with evidence of atrioventricular block (increased P–R interval) as well as a broadening of the QRS complex (*A*). Heart rate (*B*) was measured in 4 transgenic mice and 10 nontransgenic littermates, which were derived from a total of 3 litters. The mothers of these mice had received captopril through the drinking water from day 12 of pregnancy, as described.

QRS complex in the ECG of transgenic mice (Fig. 4*A*) suggests a decrease in conduction velocity in the ventricles of transgenic mice.

DISCUSSION

The data from this study have demonstrated that the AT_1 receptor expressed in cardiac myocytes mediates important, direct-growth responses. When overexpressed early in embryonic development, this receptor mediates myocyte hyperplasia leading to massive atrial enlargement. Of significant interest is the lethal phenotype associated with disturbances in heart rate and atrioventricular conduction. These results support the importance of local Ang II and AT_1 receptors in cardiac development and in the pathophysiology of cardiac disease.

Several lines of evidence suggest that Ang II can function as a growth factor for cells of the cardiovascular system. In vascular smooth muscle cells, Ang II can induce the expression of several protooncogenes as well as the expression of several growth factors, resulting in the induction of hypertrophic or hyperplastic growth during development as well as in several pathophysiologic conditions, such as hypertension-induced vascular hypertrophy and, at least in rodents, the vascular response to injury (16–18). Experimental evidence also suggests that Ang II induces the growth of cardiac cells; however, neither the mechanism nor the pathway is as clearly defined. *In vivo*, Ang II infusion can induce cardiac hypertrophy even when the pressor activity of Ang II is blocked (19), or when subpressor doses of Ang II are used (20). Moreover, both ACE inhibitors and AT_1 receptor antagonists can blunt pressure overload cardiac hypertrophy and increase survival by an

action not entirely explainable by a reduction in hemodynamic stress (21, 22). In culture, both myocytes and nonmyocytes have been reported to express various components of the renin–angiotensin system as well as the angiotensin receptors (6), and treatment of cultured cardiac myocytes with Ang II has been shown to lead to an induction of cellular hypertrophy (23–25). However, it is unclear whether this is a direct action of Ang II on the myocyte or an indirect action of Ang II that is mediated by nonmyocyte contamination. Indeed, treatment of enriched nonmyocyte cultures with Ang II results in the induction of growth factor expression that may affect the myocytes in a paracrine manner (26–29). Thus, it is possible that the actions of Ang II on the cardiac myocyte are indirect and are mediated through the nonmyocyte cells.

In this report, we have employed a transgenic approach to determine whether the AT_1 receptor alone in cardiac myocytes is sufficient to induce cardiac growth. To overexpress AT_1 receptors selectively in cardiac myocytes *in vivo*, the promoter of the cardiac αMHC gene was used. In the mouse, expression of the endogenous αMHC is restricted to cardiac myocytes and shows a developmental pattern of expression in the different chambers of the heart (30). Transgenic studies using the αMHC promoter to drive expression of various genes (10, 31, 32–35) have demonstrated tissue and cell specificity of this promoter to direct expression of a transgene selectively to cardiac myocytes. The αMHC gene is expressed in the heart at low levels during embryonic development but is strongly activated in the ventricles immediately after birth (30). That overexpression of AT_1 receptors under control of the αMHC promoter leads to an increase in cardiac mass at birth indicates that the activity of the αMHC promoter is sufficient to induce cardiac growth via AT_1 receptor signaling during embryonic development. The disproportionate effect of the transgene on atrial enlargement without a clear ventricular phenotype is probably due to expression of the αMHC in the inflow tract of the developing heart (15). In the developing rat heart between embryonic days 12 and 18, αMHC gene expression is restricted to atria, sinus venosus, and the sinus horns, with only a few myocytes expressing αMHC in the ventricles and outflow tract of the heart (15). Because the murine αMHC promoter in the ventricles becomes activated only after birth, one would predict that those transgenic mice that survive longer would develop ventricular enlargement as well.

It is of interest to note that the atrial enlargement was due to hyperplasia rather than hypertrophy of cardiac myocytes, suggesting that Ang II via activation of the AT_1 receptor can act as a potent mitogen in cardiac myocytes that are not terminally differentiated. In cell culture, Ang II was strictly hypertrophic to normal neonatal cardiac myocytes, whereas it was mitogenic to cardiac nonmyocytes (5). However, Ang II treatment of AT-1 cells (a permanent cell line expressing the simian virus 40 (SV40) T antigen under the control of the ANP promoter) resulted in a mitogenic response (24, 36). A similar phenotype (i.e., myocyte hyperplasia) was observed in transgenic mice with cardiac overexpression of calmodulin (37), c-*myc* (38, 39), or SV40 T antigen (40) during development. In these transgenic models, the promoters (Rous sarcoma virus enhancer, atrial natriuretic factor promoter, αMHC promoter) that were used to control transgene expression were active during the embryonic period. Thus, during embryonic development, growth stimuli result in a hyperplastic response in cardiac myocytes *in vivo*. Although angiotensin AT_1 and α_{1B}-adrenergic receptors can couple to similar signaling pathways, transgenic overexpression of the α_{1B}-adrenergic receptor under control of the αMHC promoter did not result in myocyte hyperplasia but caused ventricular hypertrophy in adult mice (33). Because the α_{1B}-receptor used in these studies was a constitutively active receptor mutant, it would be interesting to test whether overexpression of a nonmutated α_{1B}-receptor would result in a different cardiac phenotype.

The finding that AT_1 receptor stimulation of embryonic cardiac myocytes *in vivo* results in cardiac hyperplasia may also be relevant to congenital cardiac disease in humans. In neonates and young infants with heart failure due to a large left-to-right shunt or with congenital coarctation of the aorta (41, 42), treatment with angiotensin-converting enzyme inhibitors has a clear beneficial effect. Activation of the renin–angiotensin system may be involved in the adaptive growth responses of the heart with congenital cardiac malformations (43).

The influence of Ang II on cardiac rhythm and conduction has been suggested from previous *in vivo* and *in vitro* studies. In isolated rat heart trabeculae, Ang II increases intracellular resistance and reduces the conduction velocity (44, 45). The presence of endogenous AT_1 receptors has been demonstrated in the rat cardiac conduction system (46–48). These observations suggest that the bradyarrhythmia observed in transgenic mice may be the result of a direct effect of the transgene on the cardiac conduction system, although the effect of the enlarged atria may also play a contributing role. It is interesting to note that transgenic mice that develop atrial enlargement due to overexpression of the SV40 T antigen display irregular heart rhythms but have a normal life span (49). Based on these observations, we postulate that the lethal effect of the αMHC-AT_1 transgene is related to bradyarrhythmia and heart block that may be particularly severe in those mice containing high transgene copy numbers and may lead to early fatality.

Specificity of the AT_1 transgene was demonstrated by increased transmission rate and prolonged survival of transgenic offspring from line M4/7 after treatment of mothers with captopril or losartan. This suggests that enhanced AT_1 signaling rather than a positional effect of the transgene insertion into the host genome is responsible for the lethal cardiac phenotype. The transmission rate of the M4/7 transgene never increased above 8%, even when the pregnant mothers were treated with captopril or losartan (see Fig. 1*C*). This is significantly lower than the expected transmission rate of 50% for a transgene that was inserted into the genome of the fertilized embryo at the one-cell stage. This low transmission rate of the transgene is probably related to the mosaicism of the founder mice. However, residual activity of the transgenic AT_1 receptor due to insufficient concentrations of captopril and losartan in the fetuses and in the milk cannot be ruled out.

In summary, the data demonstrate that overexpression of the AT_1 receptor in atrial myocytes during the embryonic period results in myocyte hyperplasia, leading to massive atrial enlargement at birth. Newborn mice are severely bradycardic and die within the first weeks after birth. This transgenic model demonstrates the potency of Ang II as a modulator of cardiac growth and may help to further identify the signaling pathways and cell-cycle regulation that underlie Ang II's effect on the heart. Although this does not rule out the possibility of additional, indirect actions or influences on cardiac growth, these results strongly support the notion that the renin–angiotensin system can exert direct stimulator effects on cardiac myocytes.

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