MECHANISMS OF SIGNAL TRANSDUCTION:

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DIRECT AND INDIRECT EFFECTS OF ANGIOTENSIN II ON THE HEART*

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Jorge P. van Kats‡§‖, Danielle Methot§¶, Pierre Paradis‡, David W. Silversides, and Timothy L. Reudelhuber‡ ‡‡**

*From the ‡Laboratory of Molecular Biochemistry of Hypertension, Clinical Research Institute of Montréal, Québec H2W 1R7, Canada, ¶Department of Pathology, University of Washington, Seattle, Washington 98195-7335, and **Centre de recherche en reproduction animale, Faculté de Médecine Vétérinaire de l'Université de Montréal, Sainte Hyacinte, Québec J2S 7C6, Canada*

Angiotensin II is a peptide hormone regulator of blood pressure and fluid balance in mammals. Evidence obtained largely *in vitro* has also suggested that angiotensin II has growth-promoting effects and that it might thereby contribute to such pathological phenomena as cardiac hypertrophy, a major risk factor for cardiovascular mortality. It has been difficult to test for the direct growth-promoting effects of angiotensin II *in vivo*, however, because of the generalized effects of the peptide on hemodynamics. To overcome this limitation and to test for cardiac-specific functions of angiotensin II, we generated transgenic mice expressing an angiotensin II-producing fusion protein exclusively in cardiac myocytes. Our findings are the first to distinguish between local and systemic effects of angiotensin II on the heart and introduce a novel technique for studying tissue-specific peptide function.

The circulating renin-angiotensin system (RAS)[1] is a key regulator of blood pressure and volume homeostasis in mammals, and its peptide product, angiotensin II (Ang II), has been implicated in many of the harmful sequelae of high blood pressure, including cardiac and vascular hypertrophy and renal injury. In recent years, it has been suggested that Ang II could also have direct effects on the heart, including facilitating sympathetic nerve transmission, increasing cardiac contractility, and initiating structural remodeling resulting in cardiac hypertrophy and fibrosis (1–4). Although inhibition of the cardiac RAS in these pathologies has shown some therapeutic benefit (5–8), it has been impossible to determine whether this benefit is secondary to reducing cardiac workload or whether it is due to inhibition of the local actions of Ang II on cardiac cells. These questions are the subject of much current debate because of their importance in determining treatment targets in patients with increased risk for cardiac disease (9).

Animal models resulting from localized overexpression of RAS components (10–13) have not resolved this issue either because overexpression of the enzymes involved in Ang II synthesis does not automatically result in increased cardiac Ang II formation or because Ang II production is not limited to the heart. At present, no animal model has been reported with localized genetic inactivation of RAS components.

To test for tissue-specific functions of Ang II *in vivo*, we developed a fusion protein that can direct the release of Ang II peptide within specific target tissues of transgenic animals (14, 15). Here we report on the targeted expression of this fusion protein specifically in the mouse heart, where it leads to high local production and secretion of Ang II by cardiomyocytes. Our approach is the first demonstration of *in vivo* genetic targeting of biological active peptides to a specific organ and allows for the first time discrimination between the local (direct) and systemic (indirect) effects of Ang II on the heart.

MATERIALS AND METHODS

Construction of Expression Vectors and Production of Transgenic Mice—The vectors encoding the Ang II-releasing fusion protein and the frog skin Ang II-releasing fusion protein (fsAng II), a peptide structurally related to Ang II (16), have been described previously (14, 15). Expression of the fusion protein in the mouse heart was driven by a 6-kB fragment of the mouse alpha myosin heavy chain gene promoter (a generous gift from Jeffrey Robbins, University of Cincinnati) cloned upstream of the fusion protein cDNA. FVB/N mouse embryos were manipulated according to standard protocols for pronuclear microinjection (17), and all subsequent breeding was carried out in the FVB/N line. A plasmid containing the tyrosinase gene was co-injected with the fusion protein construct to allow easy identification of TG mice by coat color (18). Male transgenic mice were tested with their non-transgenic littermates at 5–6 weeks or 10–12 weeks of age. Animals were handled in accordance with institutional guidelines.

Physiological and Biochemical Characterization of Transgenic Mice—Systolic blood pressure and heart rate of conscious mice were measured by tail cuff plesmythography using the Visitech Systems BP-2000 (Apex, NC) (19). Cardiac hypertrophy was quantitated by measurement of ventricular weight and by calculating the heart weight-to-body weight ratio and heart weight-to-tibia length ratio.

For measurement of angiotensin peptide in plasma and heart, blood was collected via cardiac puncture of anesthetized mice (2.5 mg of ketamine and 0.06 mg of acepromazine) in the presence of inhibitor solution (13). Plasma was separated at 4 °C and stored at −80 °C. Hearts were taken out, blotted on paper, and freed from atria. Ventricles were immediately frozen in liquid nitrogen. Angiotensin peptides were extracted from plasma and tissue (13, 20) and assayed for Ang II or fsAng II by radioimmunoassay (21). The antiserum displayed 75–85% cross-reactivity with fsAng II and 100% with the Ang II metabo-

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‡‡ To whom correspondence should be addressed: IRCM 110 Pine Ave. W., Montréal QC H2W 1R7, Canada. Tel.: 514-987-5716; Fax: 514-987-5717; E-mail: reudelt@ircm.qc.ca.

[1] The abbreviations used are: RAS, renin-angiotensin system; Ang II, angiotensin II; fsAng II, frog skin Ang II; HPLC, high performance liquid chromatography; TG, transgenic.

lites Ang III and Ang IV (Ang-(2–8) and Ang-(3–8), respectively). Active plasma renin concentration was determined in plasma using an enzyme kinetic assay for Ang I generation in the presence of excess sheep renin substrate (20).

To measure angiotensin receptor densities (22), crude cardiac plasma membranes were prepared from wild type ($n = 4$) and Ang II-producing mice (1B, $n = 4$). Competitive binding assays were performed by using increasing concentrations (10^{-12}-10^{-6} M) of [Sar^1, Ile^8]Ang II and 40–50 pM ^{125}I-[Sar^1, Ile^8]Ang II (2200 Ci/mmol). Binding data were analyzed with EBDA-Ligand software (Biosoft, Milltown, NJ).

High Performance Liquid Chromatography of Plasma and Tissue Extracts—Extracts from plasma or cardiac tissue were injected with a tracer amount of ^{125}I-Ang II on a reversed-phase HPLC system using a μBondapak C18 steel column of 150 × 3.9 mm with a 10-μM particle size (Waters, Milford, MA). Angiotensin peptides were separated according to a modification of a protocol described previously (20). As a reference for retention times for fsAng II, Ang II, and its metabolites Ang III and IV (all from Bachem, Torrance, CA), 200–300 pg of each peptide was injected separately with a tracer amount of ^{125}I-Ang II. Fractions were neutralized with 50 μl of 0.17 M NaOH and lyophilized before radioimmunoassay.

Isolated Perfused Heart—Perfusion of isolated mouse hearts was performed to test for the cardiac release of Ang II by transgenic mice (1B and 2A; $n = 4$) and control ($n = 5$). Mice were heparinized and anesthetized (methoxyflurane), and then the heart was removed and placed in ice-cold Tyrode's buffer (23). The aorta was cannulated for retrograde perfusion in a Langendorff setup. Hearts were perfused at a constant flow of 2 ml/min with oxygenated Tyrode's buffer. Coronary effluent was collected for 25 min in a tube on ice following a 10-min stabilization period. Perfusate was concentrated on Sep-Pak C18 cartridges (Waters, Milford, MA), and eluate was assayed for angiotensin content after lyophilization.

Histochemistry—Hearts were collected for histology after *in situ* fixation in Bouin's fixative and embedded in paraffin. Serial cuts of 5 μM were prepared and stained with Sirius Red to evaluate collagen content (13). Fibrosis was quantitated by recording 10 randomly chosen fields without arteries (magnification ×400) from the left ventricular free wall of three non-consecutive transversal sections per heart. Sirius Red staining on sections from Ang II-producing (1B, $n = 6$) and control ($n = 4$) mice was analyzed using a digital imaging system (Empix Imaging, Mississauga, Canada).

Hypertensive Challenge—Osmotic minipumps (Alza Corp., Palo Alto, CA) were implanted subcutaneously in anesthetized (2.5 mg of ketamine and 0.06 mg of acepromazine) transgenic (1B, $n = 9$) and control ($n = 9$) mice, aged 12 weeks, for the administration of Ang II (350 ng/kg/min) or saline. Blood pressure was measured for 2 weeks, after which mice were sacrificed and hearts were collected for histology and assessment of heart weight.

Statistical Analysis—All values are expressed as mean ± S.E. Statistical analysis of tissue angiotensin levels was performed by one-way ANOVA and Dunnet's post-hoc *t* test. Statistical significance was set at $p < 0.05$.

RESULTS

Expression of Fusion Protein in Vivo—We generated mice in which the cardiomyocytes chronically release Ang II by making them transgenic for an Ang II-releasing fusion protein under the control of the alpha myosin heavy chain promoter. The essential features of this fusion protein (15) are as follows. To ensure cell secretion of the protein, a signal peptide from human prorenin is linked to a portion of the mouse heavy chain constant region of IgG2B, which mainly provides mass to the protein (Fig. 1*a*). The Ig fragment is linked to the peptide of interest by a molecular spacer, a portion of the human prorenin prosegment, containing a cleavage site at its carboxyl terminus for the protease furin. After cleavage, the encoded peptide is released in the secretory pathway and secreted by the cell. The same approach was followed for localized delivery of fsAng II. This peptide, which is structurally related to Ang II (Fig. 1*b*), has similar pressor and receptor binding properties to native Ang II (14, 16). The advantage of fsAng II over Ang II is that the proline in the amino-terminal extension of fsAng II can act as a block to degradation of the peptide by aminopeptidases (24). We established three independent transgenic lines producing the Ang II-releasing fusion protein (designated 1A, 1B, and 1C) and five lines producing the fsAng II-releasing fusion protein (2A, 2B, 2C, 2DA, and 2DB). In all lines, the transgene was transmitted in a Mendelian fashion (data not shown). Here we present the data obtained with three of these lines of mice (1B, 2A, and 2C), the results of which were reproduced in the other lines (data not shown). Expression of the fusion protein in line 2A led to the highest cardiac levels of angiotensin peptide (roughly 6000 times the levels in control mice), whereas we found intermediate and moderately elevated angiotensin peptide levels in lines 2C and 1B, respectively (Table I).

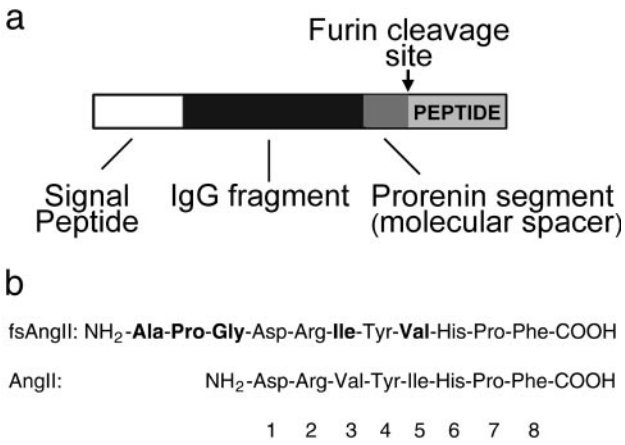

FIG. 1. **Fusion protein and its released peptide products.** *a*, structural components of the engineered fusion protein. *b*, peptide structures of fsAng II and Ang II. The amino-terminal extension and the 3′ and 5′ inversion of valine and isoleucine in the fsAng II structure are printed in *bold*.

Because angiotensins have been shown to be rapidly degraded in the interstitial space (25), we wanted to ascertain that the Ang II we measured in the heart was intact Ang II peptide and not its metabolites. Therefore, we analyzed cardiac extracts from line 1B by HPLC. Following radioimmunoassay of the HPLC fractions, we found a single peak corresponding to Ang II but not its metabolites (Fig. 2, *a* and *b*). Because recovery in our HPLC-separated fractions was >80%, our results suggest that immunoreactive angiotensin in transgenic hearts from the line with the lowest expression corresponds to intact Ang II and not its metabolites. The release of angiotensin peptide from the heart into the circulation was evident in the line with the highest expression of cardiac fsAng II (2A; Table I). Further proof for spillage of Ang II from the heart in the two lines expressing fsAng II comes from the measurements of active plasma renin concentration. Elevated Ang II levels in the circulation suppress (via a negative feedback on renal juxtaglomerular cells) the formation and secretion of renin from these cells. Renin activity was suppressed in lines 2A and 2C, whereas in line 1B it was not affected (Table I). Indeed, HPLC analysis of plasma from line 2A displayed a single Ang II immunoreactive peak, which approximates the retention time of fsAng II and not Ang II (Fig. 2*c*). The small difference between the retention times of the fsAng II standard and the peptide derived from plasma extract suggests that the angiotensinase-resistant des-[Ala^1]-fsAng II is the main form of the fsAng II peptide in the circulation. This result also demonstrates that endogenous Ang II production was largely suppressed in line 2A and that virtually all of the circulating Ang II (fsAng II) was derived from the hearts of these animals.

Although we did not find any evidence for Ang II leakage into the plasma from the hearts of 1B mice, mounting the hearts in a Langendorff preparation showed that coronary perfusate collected during 25 min from hearts obtained from transgenic

TABLE I

Characterization of transgenic and control mice at 5–6 and 10–12 weeks of age

Data are collected from non-transgenic mice (control) and transgenic Ang II-producing (1B) or fsAng II-producing (2A,2C) mice. HW/BW, heart weight-to-body weight ratio; HW/TL, heart weight-to-tibia length ratio; bpm, beats per minute.

	Control	1B (Ang II)	2A (fsAng II)	2C (fsAng II)
Age 5–6 weeks	$n = 15$	$n = 6$	$n = 9$	$n = 8$
BW (g)	21.6 ± 0.5	21.1 ± 0.8	22.0 ± 2.2	21.2 ± 0.5
HW (mg)	91 ± 1	86 ± 3	155 ± 4[a]	95 ± 3
HW/BW (mg/g)	4.2 ± 0.1	4.1 ± 0.1	7.1 ± 0.5[a]	4.5 ± 0.1
Tibia length (mm)	16.1 ± 0.1	16.4 ± 0.2	15.8 ± 0.3	16.1 ± 0.1
HW/TL (mg/mm)	5.6 ± 0.1	5.2 ± 0.1	9.8 ± 0.1[a]	5.9 ± 0.2
Systolic pressure (mm Hg)	135 ± 4	144 ± 3	169 ± 5[b]	141 ± 2
Heart rate (bpm)	637 ± 13	677 ± 11	620 ± 11	653 ± 23
Plasma (fs)Ang II (pg/ml)	126 ± 25	290 ± 97	443 ± 103[a]	72 ± 8
Cardiac (fs)Ang II (ng/g)	0.23 ± 0.04	12.4 ± 1.6[b]	1384 ± 123[b]	219 ± 15[b]
Renin (ng Ang I/ml/h)	1167 ± 136	752 ± 258	9.3 ± 42[b]	157 ± 23[b]
Age 10–12 weeks	$n = 18$	$n = 11$	$n = 8$	$n = 13$
BW (g)	24.8 ± 0.4	23.2 ± 0.6	26.4 ± 0.7	27.2 ± 0.9
HW (mg)	98 ± 2	92 ± 3	153 ± 5[b]	121 ± 6[b]
HW/BW (mg/g)	4.0 ± 0.1	4.0 ± 0.1	5.3 ± 0.1[b]	4.2 ± 0.1[c]
Tibia length (mm)	17.4 ± 0.1	16.8 ± 0.3	17.8 ± 0.2	17.9 ± 0.2
HW/TL (mg/mm)	5.8 ± 0.1	5.6 ± 0.1	8.7 ± 0.3[b]	6.7 ± 0.3[b]
Systolic pressure (mm Hg)	131 ± 3	137 ± 6	163 ± 6[b]	154 ± 4[a]
Heart rate (bpm)	681 ± 18	619 ± 18	677 ± 12	737 ± 16
Plasma (fs)Ang II (pg/ml)	127 ± 14	77 ± 8	340 ± 21[a]	131 ± 31
Cardiac (fs)Ang II (ng/g)	0.19 ± 0.06	5.7 ± 0.7[b]	1123 ± 44[b]	225 ± 42[b]
Renin (ng Ang I/ml/h)	1646 ± 194	1526 ± 244	83 ± 24[b]	457 ± 137[b]

[a] $P < 0.01$.
[b] $P < 0.001$ transgenic *versus* control.
[c] $P < 0.05$.

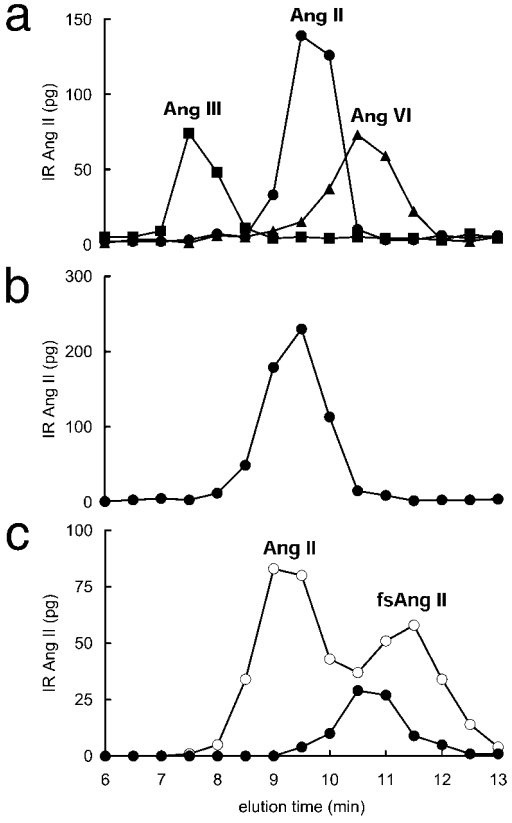

FIG. 2. **Characterization of peptide content.** *a*, HPLC elution profiles of Ang II (*circles*), Ang III (*squares*), and Ang IV (*triangles*) peptide standards. *b*, representative example of a cardiac extract from an Ang II-producing mouse. *c*, Ang II and fsAng II peptide standards (*open circles*) and plasma extract from a fsAng II-producing mouse (*closed circles*). *IR*, immunoreactive.

mice (1B and 2A) contained 30–120 pg of Ang II and 400–2700 pg of fsAng II, respectively. Perfusate collected from the hearts of non-transgenic mice did not contain any detectable Ang II immunoreactivity, which is in agreement with a previous study using isolated perfused hearts of normal rats (23). These results confirm that even though expression of our Ang II-releasing fusion protein in cardiomyocytes does not result in detectable increases in plasma levels of Ang II, the cardiac cells do release the peptide *in vivo*.

Physiological Characterization of Transgenic Mice—To assess and compare the physiological effects of elevated cardiac (all lines) and combined cardiac and circulating (2A and 2C) Ang II levels, we measured systolic blood pressure and quantitated cardiac hypertrophy in transgenic mice and control littermates. The abundant overflow of fsAng II from the heart into the circulation led to the elevation of blood pressure in transgenic mice from line 2A at 5–6 and 10–12 weeks and in transgenic mice from line 2C at 10–12 weeks (Table I). We did not observe any alteration of systolic blood pressure in mice from line 1B as compared with control mice, and no differences were seen in heart rates between transgenic and non-transgenic mice. Increase of systolic blood pressure in line 2A at 5–6 and 10–12 weeks of age was accompanied by cardiac hypertrophy as indicated by an increase of heart weight-to-body weight ratio and heart weight-to-tibia length ratio (Table I). These hypertrophic indices were only elevated in mice of line 2C at 10–12 weeks of age, when they had developed full hypertension. We found no evidence of cardiac hypertrophy in mice from line 1B despite dramatically increased cardiac Ang II content. Systolic blood pressure in transgenic mice correlated with the degree of cardiac hypertrophy as determined by heart weight-to-body weight ratio (Fig. 3) at 5–6 weeks ($R^2 = 0.53$, $p < 0.001$) and at 10–12 weeks ($R^2 = 0.32$, $p < 0.01$). We also calculated significant correlations for systolic blood pressure and heart weight or heart weight-to-tibia length ratio (data not shown).

To determine whether increases in cardiac Ang II content also led to morphological changes, we examined collagen deposition by Sirius Red staining of histological sections of hearts from transgenic mice. We observed a thickening of left ventricular wall and interventricular septum (2A) and clearly increased perivascular and interstitial fibrosis (2A, 2C) in hypertrophied hearts from hypertensive transgenic mice (Fig. 4,

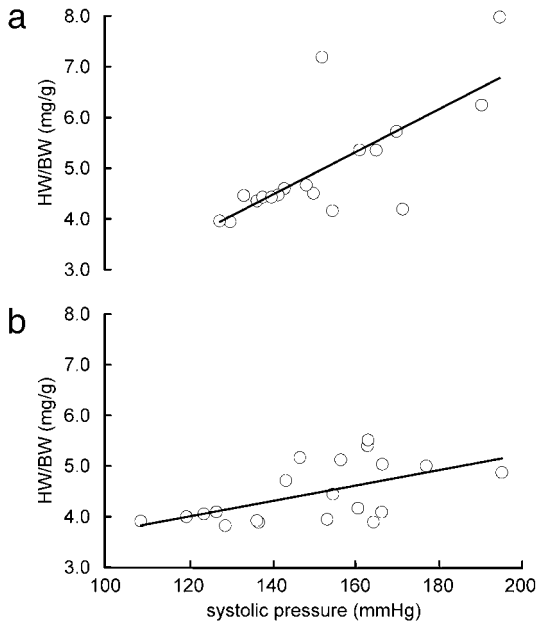

FIG. 3. **Correlation between heart weight-to-body weight ratio (*HW/BW*) and systolic blood pressure of transgenic mice (data from all lines) at 5–6 weeks (*a*) and at 10–12 weeks (*b*).**

a–h). Interestingly, we also observed a significant increase in interstitial fibrosis in hearts from normotensive Ang II-producing mice (1B). Analysis of Sirius Red staining from these sections revealed a 38% augmentation of collagen density ($p < 0.05$) as compared with normal mice at 10–12 weeks (Fig. 4, *i–k*). This finding demonstrates that Ang II in the heart directly stimulates the deposition of collagen in the cardiac interstitium.

To rule out the possibility that the absence of cardiac hypertrophy in the Ang II-producing mice (1B) could be due to a decrease in cardiac angiotensin receptor density as a result of chronic local elevations in Ang II, we carried out radioligand binding assays on membrane preparations from non-transgenic and transgenic mice (1B). In normal mice, we found an angiotensin receptor density of 22.5 ± 0.3 fmol/mg of protein. The binding of ^{125}I-[Sar^1, Ile^8]Ang II could largely (>95%) be blocked by 10^{-6} mol/liter losartan (specific angiotensin II type 1 receptor (AT_1) antagonist) but not by 10^{-6} mol/liter PD123319 (specific angiotensin II type 2 receptor ligand), indicating that the AT_1 receptor is the major receptor type that is present in the mouse heart. In cardiac membranes from Ang II-producing (1B) mice, we did not observe a significantly different receptor density (21.5 ± 1.9 fmol/mg of protein) nor did we detect any differences in receptor affinities (data not shown).

Hypertensive Challenge—To further test whether the mice with only local cardiac increases in Ang II (1B) had lost the ability to mount a cardiac hypertrophic response to Ang II, we implanted transgenic and non-transgenic littermates with osmotic minipumps releasing either saline or Ang II during 2 weeks. We then collected the hearts of these mice, weighed them, and prepared sections for histochemistry. Systemic administration of Ang II by osmotic minipumps increased systolic blood pressure to the same extent in Ang II-producing mice (1B) and non-transgenic controls, and the rise in systolic blood pressure was accompanied by a similar elevation of heart weight-to-body weight ratio in both groups (Fig. 5, *a* and *b*). We examined Sirius Red-stained cardiac sections and observed severe perivascular fibrosis extending into the surrounding tissue and development of interstitial fibrotic lesions in both hypertensive groups (Fig. 5, *c–f*). In the saline-treated groups, we did not see any alteration in systolic blood pressure, heart weight, or cardiac fibrosis. Thus, elevated cardiac Ang II production did not have any additional effects in mounting a response to the actions of increased circulating Ang II levels.

DISCUSSION

In the present study we have used a novel technique to differentiate for the first time between the local and peripheral effects of chronic elevations of Ang II on cardiac pathologies. To do this, we have made use of transgenes that express Ang II-producing fusion proteins in cardiomyocytes. Intracellular cleavage of the fusion protein in the secretory pathway by the protease furin leads to the secretion of excised Ang II into the cardiac interstitial space. *In vivo*, the formation of the peptides in the heart was evidenced by intact cardiac Ang II and fsAng II levels that are 20–40- and 600–6000-fold higher, respectively, than the normal cardiac Ang II level. The substantially different angiotensin peptide levels between Ang II- and fsAng II-producing hearts might be explained by the proline in the amino-terminal extension of fsAng II, which could protect this peptide against rapid degradation by aminopeptidases (24). The different cardiac angiotensin levels between transgenic lines were also reflected by the degree of leakage of the peptide from the cardiac interstitial space into the circulation and its subsequent effect on systolic blood pressure. The mice with the highest cardiac overflow of angiotensin peptide (2A) had already developed hypertension at 5–6 weeks (the earliest age at which we could reliably measure blood pressure). In the line with moderate spillage (2C), the increase of systolic blood pressure was more modest and became apparent only in older mice (10–12 weeks). In mice expressing the Ang II-producing fusion protein (1B), the amount of Ang II leaking from the heart into the circulation was insufficient to affect either plasma Ang II levels or systolic blood pressure.

Ventricular enlargement in transgenic mice was observed in the hypertensive lines 2A and 2C and only in line 1B following the implantation of Ang II-releasing minipumps. The augmentation of ventricular mass in these lines appears to be a consequence of the peripheral actions of Ang II rather than a result of its direct growth-promoting effect on cardiomyocytes. The inability of Ang II to directly stimulate cardiac growth is best illustrated by lines 2C and 1B. The fsAng II-producing mice of line 2C slowly develop hypertension, which only becomes significantly different from controls at 12 weeks in parallel with all the indices for cardiac hypertrophy. We could not detect any significant indication of cardiac hypertrophy at 5–6 weeks even though these animals have cardiac fsAng II levels similar to those seen at 12 weeks. Normotensive Ang II-producing mice (1B) have 20–40-fold elevated cardiac Ang II levels as compared with control without altered ventricular weights. One might argue that these mice have become somehow insensitive for the action of Ang II. However, the number of angiotensin receptors found on cardiac cells was not changed, and the augmented interstitial fibrosis demonstrates that the cardiac Ang II is indeed active.

Recently, some reports appeared that aimed to study the cardiac effects of Ang II by means of targeted overexpression of RAS components in the mouse heart (10–13). In all these models, cardiac hypertrophy was observed; however, this was not necessarily associated with cardiac Ang II signaling. Ventricular enlargement was accompanied with hypertension (13) or leakage of RAS components into the circulation (11), thereby interfering with the systemic RAS. Our findings seem to be in disagreement with the observation that overexpression of the murine AT_{1a} (10) or human AT_1 receptor (12) in the hearts of transgenic mice led to increased cardiac signaling of the AT_1

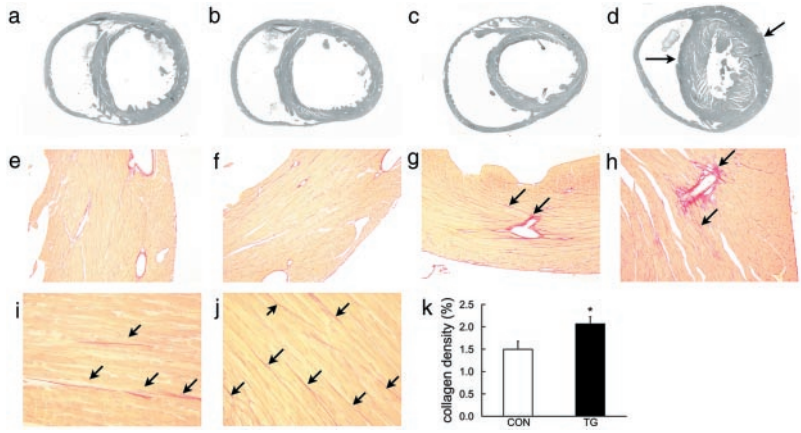

FIG. 4. **Evaluation of cardiac morphology.** The two upper rows show representative photomicrographs of cardiac sections from control (*CON*) and transgenic (*TG*) mice at 10–12 weeks. Transverse sections of whole heart (*a–d*) (magnification ×25) and portions of the left ventricular free wall (*e–h*) (magnification ×100) from control mice (*a* and *e*), Ang II-producing transgenic mice (line 1B) (*b* and *f*), and fsAng II-producing transgenic mice (line 2C) (*c* and *g*) (line 2A) (*d* and *h*). *Arrows* indicate the thickened left ventricular free wall and interventricular septum (*d*) and increased perivascular and interstitial fibrosis (*g* and *h*). *Bottom row*, evaluation of interstitial fibrosis. *Photomicrographs*, representative examples of left ventricular fields from control (*i*) and transgenic mice (*j*) (line 1B) used for digital imaging analysis (magnification ×400). *Arrows* indicate interstitial collagen fibers. *Bar graph*, analysis of cardiac collagen density in control (*CON*, *open bar*) and transgenic mice (*TG*, *solid bar*) (*k*). *, $p < 0.05$ transgenic *versus* control.

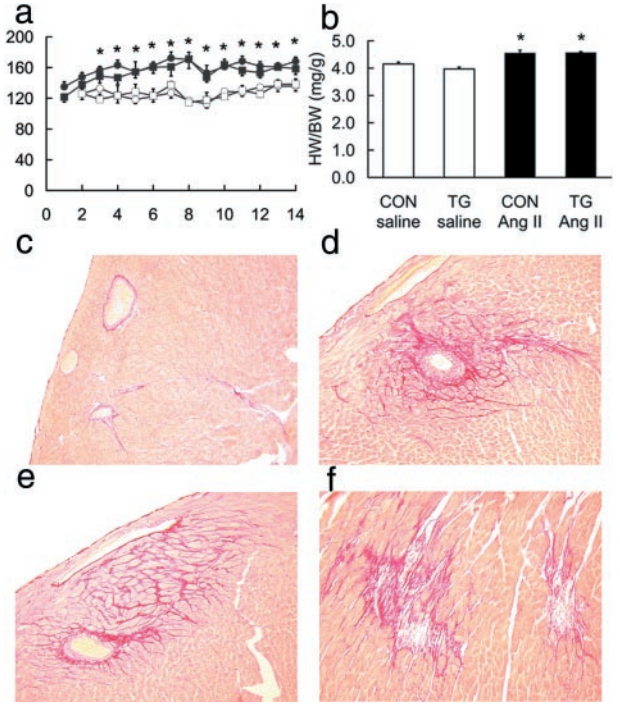

FIG. 5. **Effects of implantation of Ang II- or saline-releasing osmotic minipumps in control (*CON*) and transgenic (*TG*) mice.** *a*, *line graph*, systolic blood pressure in transgenic (*squares*) and control mice (*circles*) after saline (*open symbols*) or Ang II infusion (*closed symbols*). *, $p < 0.05$ Ang II infusion *versus* saline. *b*, *bar graph*, heart weight-to-body weight ratio (*HW/BW*) after saline (*open bars*) or Ang II infusion (*closed bars*). *c*, *photomicrographs* (magnification ×100). Little perivascular and interstitial fibrosis was observed in saline-treated transgenic mice. Following Ang II-treatment, severe perivascular and interstitial fibrosis was found in hearts from transgenic (*d*) and control (*e*) mice. *f*, an example of a fibrotic lesion found in Ang II-treated control mouse.

receptor and growth of atrial (10, 12) and ventricular (12) cardiomyocytes independent of blood pressure (12). These models mimic a state of elevated AT_1 receptor expression in the heart that may occur under pathological conditions. It has been shown that cardiac AT_{1a} receptor mRNA level and receptor density are increased following myocardial infarction (MI) in rats (26). In mice with a knockout of this receptor, ventricular remodeling was attenuated and survival was improved following MI (27). These observations suggest an involvement of increased cardiac expression of the AT_1 receptor in the development and maintenance of structural remodeling following MI. In our model, with the abundant presence of ligand in healthy mice, the number of cardiac AT_1 receptors may have been a limiting factor. Another possible explanation could be that the resulting pathologies are only apparent after an extended period of time. For example, in the mice overexpressing the human AT_1 receptor, cardiac hypertrophy only becomes apparent after 3–4 months (12), suggesting the requirement of additional conditions, such as aging, leading to the phenotype. In the Ang II-producing mice (line 1B), no signs of cardiac hypertrophy were observed even up to 4 months (data not shown). Therefore, Ang II signaling alone is not a switch that can trigger an immediate hypertrophic response in cardiomyocytes *in vivo* as opposed to the direct growth-promoting properties of the peptide observed *in vitro* (28, 29).

The structural remodeling of the heart that we observed in all hypertensive transgenic mice may have arisen from both the hemodynamic and humoral effects of Ang II (1, 30). It has been reported that cardiac AT_1 signaling is not a prerequisite in pressure overload-induced cardiac hypertrophy (31, 32) and fibrosis (32). Elevated circulating Ang II levels may also induce the production and release of hormones such as aldosterone and endothelin, which are known to influence ventricular remodeling (33–37). In normotensive Ang II-producing mice with unaltered plasma Ang II levels, we detected a significant increase in interstitial fibrosis as compared with non-transgenic controls. This observation points to a direct effect of Ang II on cardiac cells and is in agreement with earlier reports that propose Ang II-induced cardiac fibrosis independent of blood pressure (8, 12). Interstitial fibrosis without perivascular fibrosis was present in transgenic mice following overexpression of the human AT_1 receptor (12) or a constitutively active mutant of the murine Gαq subunit (38) in cardiomyocytes. These observations support the contention that the stimulation of pathways converging to activated Gαq proteins (*e.g.* Ang II, catecholamines, and endothelin) can lead to interstitial collagen deposition in the heart. Cardiac Ang II can mediate its fibrotic effects via fibroblasts and cardiomyocytes since both express the AT_1 receptor (39, 40). Ang II stimulates the proliferation of fibroblasts (41) and their production of collagen (42) and trans-

forming growth factor β(43). Recently, Ang II was also found to promote cardiac aldosterone production (44), although it is unknown in which cell type. In our transgenic mice, Ang II is produced and released by cardiomyocytes. Therefore, the interstitial fibrosis that we observed in Ang II-producing mice can result from a paracrine effect of Ang II on fibroblasts or from other mediators that are released upon autocrine stimulation of cardiomyocytes, as previously proposed by others (12, 40). The characteristics of such mediators are unidentified, although transforming growth factor β and endothelin seem to be good candidates (8, 37, 41, 45, 46).

In summary, we have developed transgenic mice that express a fusion protein leading to the synthesis and release of an Ang II peptide in cardiomyocytes. Expression of the transgene led to very high Ang II levels in the heart and release of the peptide into the circulation. When released at sufficient amounts, this circulating Ang II leads to hypertension and structural remodeling of the heart, which is characterized by ventricular enlargement and cardiac fibrosis. Elevated cardiac Ang II levels alone do not directly induce cardiac hypertrophy but do increase interstitial fibrosis. Our findings are the first to discriminate between the local and systemic effects of Ang II on the heart.

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