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Role of cardiac overexpression of ANG II in the regulation of cardiac function and remodeling postmyocardial infarction

Jiang Xu¹, Oscar A. Carretero¹, Chun-Xia Lin¹, Maria A. Cavaasin¹, Edward G. Shesely¹, James J. Yang², Timothy L. Reudelhuber³, and Xiao-Ping Yang¹

¹ Hypertension and Vascular Research Division, Department of Internal Medicine, Henry Ford Hospital and Wayne State University, Detroit, Michigan

² Department of Biostatistics and Research Epidemiology, Henry Ford Hospital and Wayne State University, Detroit, Michigan

³ Molecular Biochemistry of Hypertension, Clinical Research Institute of Montreal, Montreal, Canada

Abstract

ANG II has a clear role in development of cardiac hypertrophy, fibrosis, and dysfunction. It has been difficult, however, to determine whether these actions are direct or consequences of its systemic hemodynamic effects in vivo. To overcome this limitation, we used transgenic mice with cardiac-specific expression of a transgene fusion protein that releases ANG II from cardiomyocytes (Tg-ANG II-cardiac) without involvement of the systemic renin-angiotensin system and tested whether increased cardiac ANG II accelerates remodeling and dysfunction postmyocardial infarction (MI), whereas those mice show no evidence of cardiac hypertrophy under the basal condition. Male 12- to 14-wk-old Tg-ANG II-cardiac mice and their wild-type littermates (WT) were subjected to sham-MI or MI by ligating the left anterior descending coronary artery for 8 wk. Cardiac ANG II levels were ~10-fold higher in Tg-ANG II-cardiac mice than their WT, whereas ANG II levels in plasma and other tissues did not differ between strains. Systolic blood pressure and heart rate were similar between groups with or without MI. In sham-MI, Tg-ANG II-cardiac mice had increased collagen deposition and decreased capillary density. The differences between strains became more pronounced after MI. Although cardiac function was well preserved in the Tg-ANG II-cardiac mice with sham-MI, cardiac remodeling and dysfunction post-MI were more severe than WT. Our results demonstrate that, independent of systemic hemodynamic effects, cardiac ANG II may act locally in the heart, causing interstitial fibrosis in sham-MI and accelerating deterioration of cardiac dysfunction and remodeling post-MI.

Keywords

angiotensin II; myocardial infarction; heart failure; transgenic mice

Congestive heart failure (CHF) is a serious public health problem that affects about 5 million Americans with 550,000 newly diagnosed cases each year. The death rate remains high even when the best available treatments are used. It is generally agreed that activation of the renin-angiotensin system (RAS) plays an important pathophysiological role in the development and progression of CHF, since inhibition of ANG II (the major effector of

RAS) formation with angiotensin-converting enzyme inhibitors (ACEi) or blocking ANG II type 1 receptors with angiotensin receptor blockers (ARB) significantly improves cardiac function, regresses LV remodeling, and prolongs survival in patients with CHF (4, 13). Although a local RAS is suggested to be existed in the heart and part of the cardioprotective effects of ACEi or ARB is because of a direct inhibition on cardiac ANG II, it has been difficult to demonstrate such a direct effect.

Accumulating evidence suggests that all components of the RAS, including renin (which may be taken up from the circulation), angiotensinogen, ACE, angiotensins, and ANG II receptors, exist in the heart (1, 6, 23, 33). Furthermore, the concentration of ANG II is reportedly higher in the heart than in plasma (23). Thus, independent of its hemodynamic effect, locally synthesized ANG II may act in an autocrine and/or paracrine manner, stimulating progrowth and proinflammatory responses and causing cardiac remodeling (cardiomyocyte hypertrophy, fibroblast proliferation, and interstitial fibrosis) and dysfunction. However, in vivo it is difficult to be sure whether the hypertrophic and fibrotic effect of ANG II is because of its hemodynamic action or a direct effect on the heart.

Recently, transgenic mice overexpressing ANG II in the heart (Tg-ANG II-cardiac) have been developed by van Kats et al. (33). These mice have cardiac-specific expression of a transgene fusion protein that releases ANG II exclusively from cardiomyocytes without involvement of the RAS. Cardiac ANG II concentration in these mice is ~15- to 20-fold higher than wild-type controls (WT), but blood pressure and plasma ANG II do not differ from WT. Using an isolated heart preparation, van Kats confirmed that the coronary effluent from Tg-ANG II-cardiac mice contained high levels of ANG II, whereas ANG II immunoreactivity was not detected in WT, indicating that ANG II produced from myocytes is released in the interstitial space and responsible for the increased collagen observed in these mice under basal conditions (33). However, we know of no study testing whether increased cardiac ANG II potentiates the deterioration of cardiovascular function caused by a hemodynamic load on the heart, such as myocardial infarction (MI). Using Tg-ANG II-cardiac mice, we tested the hypothesis that increased ANG II in the heart, acting locally and without systemic effects, causes cardiac hypertrophy and fibrosis and aggravates cardiac remodeling and dysfunction post-MI compared with their WT littermates.

MATERIALS AND METHODS

ANG II transgenic mice (Tg-ANG II-cardiac)

This transgenic mouse strain, generated by van Kats et al. (33), has a transgene construct encoding a fusion peptide that releases ANG II directly from cardiomyocytes when the ANG II-containing precursor protein is cleaved by the ubiquitous natural protease furin. The protein has a signal peptide that directs it to the endoplasmic reticulum. In addition, the cleavage site for the protease, which releases the ANG II peptide, is very specific for furin, a protease ubiquitously found only in the secretory pathway. Because of this design, ANG II can only be generated in the secretory pathway and gets released from the cells in conjunction with the remainder of the fusion protein. We have verified the secretion of the components in both tissue culture and in the hearts of transgenic mice in previous studies and that this process does not require the RAS (33). Tg-ANG II-cardiac mice on a mixed FVB/N and C57BL/6 genetic background are currently being bred in our Mutant Mouse Facilities. Experimental animals were generated by mating transgene hemizygotes and nontransgenic littermates, which provides hemizygotes and nontransgenic offspring in approximately equal numbers. The Tg-ANG II-cardiac genotype was determined by PCR using genomic DNA isolated from tail biopsies as the template and primers Tim210 (5'-TTCTCATGCAACGTGAGAC-3'), Tim547 (5'-TG GTGATACAAGGGACATC-3'), Nos3A (5'-ATGGCGAAGCGTGTGAAGGCAACCAT-3'), and Nos3B (5'-

CCATTGCCAAATGTGCTGGTCACCAC-3'). The four primers are used in a single reaction. Tim210 and Tim547 amplify an ~400-bp fragment in the presence of an ANG II transgene, whereas Nos3A and Nos3B amplify an ~550-bp fragment from the WT *eNOS* gene and are used as internal amplification controls.

Animal procedures

Animals were housed in an air-conditioned room with a 12:12-h light-dark cycle, received standard chow, and drank tap water. Male mice 12–14 wk of age were anesthetized with pentobarbital sodium (50 mg/kg ip). MI was surgically induced by ligating the left anterior descending coronary artery as described previously (38). This study was approved by the Institutional Animal Care and Use Committee of Henry Ford Health System.

Experimental protocol

To determine whether high concentration of ANG II in the heart affect cardiac homeodynamics and function as well as morphology and histology, either under basal conditions or post-MI, mice were divided into 1) Tg-ANG II-cardiac sham-MI ($n = 10$); 2) Tg-ANG II-cardiac-MI ($n = 10$); 3) WT-sham-MI ($n = 11$); and 4) WT-MI ($n = 11$). The experiment was continued for 8 wk.

Measurement of blood pressure and cardiac function

Systolic blood pressure (SBP) and heart rate (HR) were measured in conscious mice using a noninvasive computerized tail-cuff system (BP-2000; Visitech, Apex, NC), as described previously (16, 35). Cardiac geometry and function were measured monthly with a Doppler echocardiographic system equipped with a 15-MHz linear transducer (model c256; Acuson, Mountain View, CA), as described previously (34, 37). Left ventricular (LV) ejection fraction (EF) and dimensions were evaluated. All studies were performed on conscious mice.

Histopathological study

After MI or sham-MI (8 wk), mice were anesthetized, and the heart was stopped at diastole by intraventricular injection of 15% KCl (50 μ l). The heart, lungs, and liver were weighed to assess hypertrophy and congestion. Organ weight was calculated as the ratio of organ wet weight (mg) to body weight (10 grams). The LV was sectioned transversely into three slices from apex to base, and the slices were rapidly frozen in isopentane precooled in liquid nitrogen and then stored at -70°C for measurements of infarct size, myocyte cross-sectional area, interstitial collagen fraction (ICF), and capillary density, as described previously (19, 36). Mice with infarct size $<20\%$ were excluded.

Immunohistochemical staining for macrophage and myofibroblast

Fresh-frozen sections of the heart (6 μ m) were fixed with acetone for 10 min and rinsed in PBS ($n = 5-6$). They were preincubated with 0.3% hydrogen peroxide in PBS to inhibit endogenous peroxidase activity and then incubated with the primary antibodies 1) rat anti-mouse CD107b (a marker for mouse macrophages, 1:500; Serotec) or 2) mouse monoclonal antibody against α -smooth muscle actin (SMA, a marker for myofibroblasts, 1:500 dilution; Sigma) supplemented with 5% of the species-respective normal serum for 1 h at room temperature. After washing with PBS, a secondary antibody (bovine anti-mouse IgG, 1:800; Santa Cruz) was applied for 30 min at room temperature, followed by avidin-biotin complex reagent (Vector) for 30 min and 3-amino-9-ethylcarbazole (Vector) for 30 s to 1 min to visualize positive staining. The negative controls were processed in a similar fashion except that they did not receive the primary antibody. Sections were counterstained with hematoxylin. Eight images of each slide were captured at $\times 400$ magnification using a

microscope (IX81; Olympus America, Melville, NY) equipped with a digital camera (DP70, Olympus America). Positive cells were recognized by reddish-brown staining in the cytoplasm. The number of microphages was counted with Microsuite Biological imaging software (Olympus) and expressed as number of cells per square millimeter of myocardium. α -SMA was expressed as the ratio of the positive-stained area to total area in each field. Data were analyzed by three investigators in a double-blind fashion using Microsuite Biological Suite software.

Plasma and tissue ANG II concentration

ANG II concentration was measured using a commercially available EIA kit after extraction and concentration. Briefly, a piece of each organ was homogenized using an inhibitor cocktail containing: 25 mM EDTA, 0.44 mM *o*-phenanthroline, 10–5 M, lisinopril, 1.12 mM pepstatin, 50 g/ml bestatin, 0.6 mM chymostatin, 6 mg/ml aprotinin, and 0.2% ethanol. Blood was collected in chilled syringes containing 0.1 ml of 10 $\times$ concentrated inhibitor cocktail/ml of blood, and plasma was separated by centrifugation. ANG II from both tissue homogenate and plasma was extracted using Bond Elut C₁₈ cartridges (Varian, CA) that had been activated with methanol and washed with water. Samples were washed again with water and ANG II eluted with methanol. The methanol was evaporated using a vacuum centrifuge (Savant), and dry samples were kept at –20°C until assay. The recovery for this procedure is ~85–90%.

Plasma renin concentration

After 8 wk of treatment, mice were anesthetized with pentobarbital sodium (50 mg/kg ip), and blood was collected in a microhematocrit tube by puncturing the retro-orbital plexus. Plasma was incubated with 250 ng sheep angiotensinogen (renin substrate) at 37°C for 30 min. Renin concentration was determined with a commercially available RIA kit and expressed as nanograms ANG I per milliliter per hour.

Data analysis

Data are expressed as means $\pm$ SE. Student's two-sample *t*-test was used to compare differences between groups either between strains or between treatments within strains. When multiple comparisons were performed, Hochberg's step-up procedure was used to adjust the *P* values (10). The type I error rate was set at 0.05.

RESULTS

Mortality

Tg-ANG II-cardiac mice tended to have a higher mortality rate compared with WT both during surgery and by the 1st wk of MI; surgical mortality was 27% (10 out of 37) for the Tg-ANG II-cardiac mice vs. 17.8% (5 out of 28) for WT, whereas at 1 wk MI, it was 59.3% (16 out of 27) for the Tg-ANG II-cardiac vs. 45.5% (10 out of 22) for WT. None of these differences was statistically significant. In both strains, the major cause of death during the 1st wk of MI was cardiac rupture. During 2–8 wk MI, only one mouse from each strain died. None of the sham Tg-ANG II-cardiac mice or WT controls died during or after the operation.

SBP, HR, body and tissue weight, and infarct size

SBP, HR, and body weight were similar between strains in all groups with or without MI. In the sham-MI groups, both LV and total heart weight were not statistically different between Tg-ANG II-cardiac and WT (Table 1). MI increased heart weight in both strains; however, the hypertrophic response was more profound in Tg-ANG II-cardiac mice than in WT (*P* <

0.01). No significant changes in liver and kidney weight were observed after MI, and infarct size was similar between strains (Table 1).

Cardiac function and remodeling

Overexpression of ANG II in the heart had no effect on cardiac function under basal conditions. EF and LV dimensions were similar between Tg-ANG II-cardiac and WT with sham-MI. MI significantly decreased EF and increased LV diastolic dimension (LVDd) in both strains (Figs. 1 and 2); however, LV dysfunction post-MI was more severe in Tg-ANG II-cardiac mice than in WT (Fig. 1, *top*). LVDd tended to be greater in Tg-ANG II-cardiac, although the difference between strain did not reach statistical significance (Fig. 1, *bottom*).

Interstitial fibrosis and capillary density

Under basal condition (sham-MI), ICF was greater in Tg-ANG II-cardiac mice than WT controls, whereas α -SMA expression, a marker for myofibroblast transformation, was not different between strains. MI increased ICF, which was more profound in Tg-ANG II-cardiac mice (Fig. 3, *top*). α -SMA expression also tended to be higher in Tg-ANG II-cardiac mice, although the difference was not statistically significant (Fig. 3, *bottom*). Capillary density was significantly lower in Tg-ANG II-cardiac mice either under basal condition or after MI (Fig. 4).

Macrophage infiltration in the heart

Infiltrating macrophages were observed in cardiac tissue in both WT and Tg-ANG II-cardiac strains (brown-stained cells in Fig. 5A). However, the number of macrophages tended to be higher in Tg-ANG II-cardiac mice under basal conditions. Macrophage infiltration was enhanced after MI, and it was markedly higher in Tg-ANG II-cardiac mice than WT controls (Fig. 5).

Tissues and plasma concentration of ANG II

Cardiac tissue ANG II levels in Tg-ANG II-cardiac mice were ~10-fold higher than their littermate controls (15.67 ± 1.98 vs. 1.75 ± 0.67 ng/g, $P < 0.001$), whereas ANG II in other tissues, including the liver, lungs, kidneys, and brain as well as in plasma, was similar to that in WT controls (Fig. 6). These findings confirm that, in cardiomyocytes, overexpression of the fusion protein resulted in the release of ANG II; and increased ANG II did not leak into the circulation. Plasma renin concentration did not differ between strains in the sham-MI groups and tended to increase similarly after MI in both strains, although this increase was not statistically significant (Table 1).

DISCUSSION

Using transgenic mice with cardiac-specific expression of a fusion protein that releases an ANG II peptide (Tg-ANG II-cardiac) independently of the RAS, we found that overexpression of ANG II in the heart caused interstitial fibrosis under basal conditions without affecting heart weight, SBP and HR. Cardiac function (as indicated by EF) was well preserved in Tg-ANG II-cardiac mice. After MI, locally generated ANG II aggravated cardiac remodeling and dysfunction. LV hypertrophy, chamber dilatation, fibrosis, and chronic ischemia (indicated by reduced capillary density) were more severe in Tg-ANG II-cardiac mice, whereas SBP, HR, and plasma renin concentration did not differ from WT controls, suggesting that these detrimental cardiac effects of ANG II are because of a direct action on the heart rather than a systemic hemodynamic effect. We also confirmed that cardiac expression of the ANG II-containing fusion protein led to the production of high levels of ANG II, since ANG II concentration was significantly higher in the heart than in

other tissues in Tg-ANG II-cardiac mice and 10-fold higher than cardiac ANG II in WT controls. Plasma ANG II levels in Tg-ANG II-cardiac mice were similar to WT, indicating that cardiac-generated ANG II did not leak in the circulation. Collectively, our data demonstrate that increased ANG II in the heart, acting locally and independent of its hemodynamic effects, causes cardiac hypertrophy and fibrosis and accelerates cardiac remodeling and dysfunction after MI.

Increasing evidence suggests that all components of the RAS, including ANG II, exist in the heart, where they regulate cardiac hemodynamics and function. Activation of the cardiac RAS is also involved in the pathophysiology of cardiac hypertrophy and heart failure (HF; see Refs. 5 and 29). Thus, independent of its hemodynamic effects, ANG II may act in both an autocrine/paracrine manner, promoting progrowth and proinflammatory responses; causing myocyte hypertrophy, interstitial fibrosis, and cardiac dysfunction (20); and contributing to the development of HF (22). The direct effect of ANG II on growth has been studied extensively in cultured cardiomyocytes and fibroblasts (18, 29), showing that ANG II increases protein synthesis and induces cardiomyocyte hypertrophy and cardiac fibroblast hyperplasia. In vivo, systemic infusion of ANG II at a subpressor dose is able to cause cardiac hypertrophy without raising SBP (15, 31). However, it is hard to tell whether this is because of a direct action of ANG II on cardiomyocytes or via systemic stimulation of the release of progrowth/proinflammation factors from noncardiomyocytes such as fibroblasts and/or inflammatory cells that in turn act on myocytes and cause cardiac hypertrophy, fibrosis, and dysfunction. Using aldosterone-salt rats (a model of low circulating ANG II), Sun et al. (30) demonstrated that tissue ANG II regulates inflammation and fibrogenesis in the heart, since the AT₁ blocker losartan attenuated these responses. Mazzolai et al. (21, 22) showed that cardiac overexpression of angiotensinogen in mice resulted in increased ANG II concentrations in the heart and caused both right ventricular and LV hypertrophy but had no effect on blood pressure. However, it is noteworthy that plasma angiotensinogen concentrations in these mice tended to be higher and plasma renin concentrations were decreased, suggesting possible leakage of angiotensinogen in the circulation. This raises the possibility that increased ANG II formation may not have been limited to the heart in their transgenic mice. Alternatively, Hein et al. (8) developed a mouse model with overexpression of the AT₁ receptor in cardiomyocytes. These mice have severe cardiac hypertrophy and remodeling associated with heart block and early death (8, 24), supporting the hypothesis that ANG II may act as a growth factor directly on myocytes via the AT₁ receptor to promote myocyte remodeling and dysfunction. In our study, using mice overexpressing a fusion peptide that releases ANG II directly from an ANG II-containing precursor protein (Tg-ANG II-cardiac; see Ref. 33), we found that the Tg-ANG II-cardiac mice had increased collagen deposition without obvious LV hypertrophy under basal conditions, which agrees with a previous study of van Kats et al. (33). The possible explanation for the observation that cardiac ANG II causes only fibrosis but not hypertrophy under physiological conditions is that hemodynamic loading (preload, afterload, or myocardial ischemia) may be the essential and necessary trigger for the hypertrophic response (27). Indeed, here we found that, in the presence of a hemodynamic load, such as MI, locally generated ANG II exaggerated LV hypertrophy, interstitial fibrosis, transformation of myofibroblasts, and cardiac dysfunction. Furthermore, these effects appeared to be independent of a systemic hemodynamic effect and did not require involvement of the peripheral RAS, since blood pressure, plasma renin, and plasma ANG II concentrations in Tg-ANG II-cardiac mice were similar to WT controls. The progrowth and profibrotic effects of ANG II generated by myocytes could be because of a paracrine action, since previous studies have demonstrated that ANG II concentration in the coronary effluent was markedly increased in Tg-ANG II-cardiac mice (33), indicating that ANG II was released in the interstitial space.

We also found that capillary density was significantly lower in Tg-ANG II-cardiac mice than WT either under basal conditions or after MI. The mechanisms responsible for the decreased number or density of capillaries are not entirely known. It could be because of 1) arteriolar constriction caused by locally increased ANG II, so-called capillary rarefaction; 2) increased reactive oxygen species (ROS) and oxidative stress secondary to local ANG II stimulation, leading to microvascular dysfunction; and 3) myocyte hypertrophy and reduced numbers of myocytes. Although we did not measure the number of myocytes per square millimeter, we could not exclude the possibility that increased levels of ANG II in the heart enhance apoptotic and/or necrotic myocyte death (17), thereby reducing the number of myocytes.

Myocyte-generated ANG II could also act in an intracrine fashion, causing myocyte hypertrophy, although the current study was not designed to address this question. Baker et al. (2) showed that intracellular transfection of ANG II in neonatal rat ventricular myocytes resulted in an increase in both protein synthesis and cell size that could not be blocked by losartan. Using cultured renal proximal tubule cells (PTC), Zhuo et al. (41) from our laboratory showed that microinjection of ANG II to PTC elicited a robust increase in cytoplasmic Ca^{2+} concentration. This effect was blocked by coinjection with losartan but not by adding losartan to the perfusate. These studies demonstrate the existence of a functional intracrine mechanism that contributes to intracellular ANG II-induced myocyte hypertrophy via the intracellular AT_1 receptor (26, 41). Taken together, this and other studies suggest that ANG II, acting locally in an autocrine/paracrine fashion, plays a crucial role in the development of cardiac hypertrophy and interstitial fibrosis as well as cardiac remodeling and dysfunction post-MI.

ANG II has also been reported to enhance chemokines, such as increasing monocyte chemoattractant protein (MCP)-1 expression, promoting infiltration of monocytes/macrophages, and releasing ROS from these inflammatory cells (9, 14, 25, 39). Persistent expression of cytokines and ROS in the heart may be largely responsible for progression of myocyte hypertrophy, LV remodeling, and ultimately cardiac dysfunction, since inhibition of ANG II formation by ACE inhibitors or blockade of the AT_1 receptor by ARB decreases LV hypertrophy and cardiovascular inflammation and also reduces nuclear factor- κB activity, MCP-1 and intercellular adhesion molecule-1 expression, and monocyte/macrophage infiltration (7, 9, 28, 32). In the present study, we found that Tg-ANG II-cardiac mice tended to have more infiltrating macrophages in the cardiac tissue under basal conditions. After MI, both the inflammatory response and development of cardiac remodeling and dysfunction were more severe in Tg-ANG II-cardiac mice than in WT controls, suggesting that ANG II acting locally in the heart activates the proinflammatory process that contributes to the development of LV remodeling and dysfunction, particularly in the presence of a hemodynamic load such as MI.

Limitations of the study are as follows. 1) Studies have demonstrated that ANG II promotes myocyte death by enhancing apoptosis and/or necrosis (3, 17). Thus it is an important issue that warrants further investigation to assess whether locally increased ANG II enhances apoptosis and/or necrosis and is responsible for some of the detrimental cardiac effects observed in Tg-ANG II-cardiac mice. 2) We did not study whether the detrimental cardiac effect of ANG II can be reversed by AT_1 blockers. Although previously published data showed that ANG II produced by myocytes is released in the interstitial space, use of an AT_1 blocker may help to confirm that the locally released ANG II acts on the AT_1 receptor and causes damage to the heart via an autocrine/paracrine action. 3) Recent studies suggest that aldosterone contributes in part to ANG II-induced molecular and cellular responses, including fibrosis (11, 12, 40). Although we do not think release of aldosterone is increased in Tg-ANG II mice under basal conditions, since systemic RAS is not activated in these

mice, it would be interesting to study the expression of mineralocorticoid receptor (MR) in the heart and whether blocked MR could attenuate cardiac fibrosis observed in Tg-ANG II mice under basal conditions.

In summary, using transgenic mice that overexpress a fusion protein leading to the synthesis and release of an ANG II peptide in cardiomyocytes, we demonstrated that elevated cardiac ANG II alone induced cardiac interstitial fibrosis under basal conditions, possibly via an autocrine/paracrine manner. We further showed that ANG II acts locally to exacerbate cardiac remodeling and dysfunction and accelerated development of HF in mice with MI without affecting systemic hemodynamics. These detrimental cardiac effects may be partially mediated by the proinflammatory action of ANG II. We conclude that local production of ANG II is functional and has a significant impact on pathophysiology and development of HF.

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Fig. 1.

Effect of overexpression of ANG II in the heart (Tg-ANG II) on ejection fraction (EF, *top*) and left ventricular diastolic dimension (LVDd, *bottom*) in mice with or without myocardial infarction (MI). *Left*: time course. *Right*: bar graph of average %change from basal. WT, wild-type littermates. $P < 0.01$ between strains with MI (#) and sham vs. MI within strains (*).

Fig. 2.

Top: representative echocardiograms showing left ventricular (LV) chamber dimensions and wall movement in mice with sham MI or MI. *Bottom:* representative histochemistry staining showing infarct size (IS) and cross-section left ventricles from mice with MI. Tg-ANG II, transgenic mice with overexpression of ANG II in the heart.

Fig. 3.

Effect of overexpression of ANG II in the heart (Tg-ANG II) on interstitial collagen fraction (ICF) and myofibroblast transformation indicated by expression of α -smooth muscle actin (α -SMA) in mice with or without MI. * $P < 0.01$ vs. sham within strains.

Fig. 4.

A: representative slides showing a lower capillary density in mice with overexpression of ANG II in the heart (Tg-ANG II) with or without MI compared with WT controls. B: quantitative analysis of myocardial capillary density. * $P < 0.01$ vs. sham within strains.

Fig. 5.

A: representative slides showing an increase in macrophage infiltration in mice with overexpression of ANG II in the heart (Tg-ANG II) compared with WT controls after MI. *B:* quantitative analysis of the no. of infiltrating macrophages in cardiac tissue. * $P < 0.01$ vs. sham within strains.

Fig. 6. Effect of overexpression of ANG II in the heart (Tg-ANG II) on ANG II concentration in various tissues (*left*) and plasma (*right*).

Table 1

Effect of ANG II overexpression in the heart (Tg-ANG II) on SBP, HR, tissue weight, PRC, and infarct size 8 wk after MI

	Wild-type Controls		Tg-ANG II-cardiac	
	Sham MI (<i>n</i> = 11)	MI (<i>n</i> = 11)	Sham MI (<i>n</i> = 10)	MI (<i>n</i> = 10)
Body wt, g	30.3±1.0	32.2±0.6	30.7±1.1	31.3±1.0
SBP, mmHg	115±2.1	110±3.9	115±4.2	111±2.5
HR, beats/min	675±17	710±16	675±19	721±9
LV, mg/10 g	30.0±0.6	35.1±1.3 [*]	31.9±0.6	40.6±1.8 ^{†‡}
THW, mg/10 g	40.0±0.8	46.9±1.8 [*]	42.4±0.7	56.3±2.7 ^{†§}
Lungs, mg/10 g	60.2±1.9	58.7±2.0	61.8±2.5	77.4±7.3 [‡]
Liver, mg/10 g	433±17.8	443±9.5	444±9.5	462±27.2
Kidney, mg/10 g	70.6±2.9	66.5±2.6	73.8±2.4	75.8±2.8 [‡]
PRC, ng ANG I · ml ⁻¹ · h ⁻¹	2.1±0.6	5.8±1.5	1.3±0.4	3.5±1.3
IS, %		29.3±1.9		28.9±2.4

SBP, systolic blood pressure; HR, heart rate; LV, left ventricular weight corrected by body weight; THW, total heart weight corrected by body weight; lungs, liver, and kidney indicate weight corrected by body weight; sham indicates sham operation; MI, myocardial infarction; PRC, plasma renin concentration; IS, infarct size.

^{*} $P < 0.01$ and

[†] $P < 0.001$ vs. sham within strains.

[‡] $P < 0.05$ and

[§] $P < 0.01$ between strains with MI.