Cardiac Angiotensin II Type 2 Receptor Activates the Kinin/NO System and Inhibits Fibrosis

Satoshi Kurisu, Ryoji Ozono, Tetsuya Oshima, Masayuki Kambe, Takafumi Ishida, Hiroshi Sugino, Hideo Matsuura, Kazuaki Chayama, Yasuhiro Teranishi, Osamu Iba, Katsuya Amano, Hiroaki Matsubara

Abstract—We have previously demonstrated that stimulation of the angiotensin (Ang) II type 2 receptor in vascular smooth muscle cells caused bradykinin production by activating kininogenase in transgenic mice. The aim of this study was to determine whether overexpression of AT\(_2\) receptors in cardiomyocytes attenuates Ang II–induced cardiomyocyte hypertrophy or interstitial fibrosis through a kinin/nitric oxide (NO)-dependent mechanism in mice. Ang II (1.4 mg/kg per day) or vehicle was subcutaneously infused into transgenic mice and wild-type mice for 14 days. The amount of cardiac AT\(_2\) receptor relative to AT\(_1\) receptor in transgenic mice was 22% to 37%. Ang II caused similar elevations in systolic blood pressure (by \(\approx 45\) mm Hg) in transgenic mice and wild-type mice. Myocyte hypertrophy assessed by an increase in myocyte cross-sectional area, left ventricular mass, and atrial natriuretic peptide mRNA levels were similar in transgenic and wild-type mice. Ang II induced prominent perivascular fibrosis of the intramuscular coronary arteries, the extent of which was significantly less in transgenic mice than in wild-type mice. Inhibition of perivascular fibrosis in transgenic mice was abolished by cotreatment with HOE140, a bradykinin B\(_2\) receptor antagonist, or L-NAME, an inhibitor of NO synthase. Cardiac kininogenase activity was markedly increased (\(\approx 2.6\)-fold, \(P<0.001\)) after Ang II infusion in transgenic mice but not in wild-type mice. Immunohistochemistry indicated that both bradykinin B\(_2\) receptors and endothelial NO synthase were expressed in the vascular endothelium, whereas only B\(_2\) receptors were present in fibroblasts. These results suggest that stimulation of AT\(_2\) receptors present in cardiomyocytes attenuates perivascular fibrosis by a kinin/NO-dependent mechanism. However, the effect on the development of cardiomyocyte hypertrophy was not detected in this experimental setting. (Hypertension. 2003;41:99-107.)

Key Words: receptors, angiotensin II • kinins • mice • fibrosis • hypertrophy

Angiotensin (Ang) II is an important humoral factor responsible for cardiomyocyte hypertrophy as well as interstitial hyperplasia.\(^1\) Both of the major Ang II receptor subtypes, AT\(_1\) and AT\(_2\), are expressed in the heart.\(^2\) The AT\(_2\) receptor (AT\(_2\)R) has been implicated in suppression of myocardial hypertrophy,\(^3\)--\(^6\) fibroblast proliferation,\(^6\),\(^7\) and vascular cell hyperplasia.\(^8\),\(^9\) However, little is known about the mechanisms by which this receptor subtype exerts such antigrowth effects and the role of the cardiac AT\(_2\)R in cardiac diseases in vivo. Previous studies have suggested possible involvement of the bradykinin/nitric oxide (NO)/cGMP system in AT\(_2\)R-mediated physiological functions in the rat aorta,\(^10\) canine coronary artery,\(^11\) a rat model of heart failure caused by myocardial infarction,\(^12\),\(^13\) hypertrophied rat heart caused by aortic coarctation,\(^4\) and in the kidney.\(^14\) We have recently demonstrated that AT\(_2\)R overexpression in vascular smooth muscle cells activates the vascular kinin system and causes vasodilation in transgenic mice.\(^15\) However, there is no direct evidence linking the AT\(_2\)R to cardiac bradykinin synthesis and activation of the NO system. It has also not been determined whether stimulation of the AT\(_2\)R and/or bradykinin has direct effects on myocyte hypertrophy and interstitial hyperplasia. Studies conducted in 2 laboratories\(^16\),\(^17\) with AT\(_2\)R-null mice showed conflicting results, the reason for which remains to be determined.

We have recently developed transgenic (TG) mice overexpressing the AT\(_2\)R selectively in cardiomyocytes.\(^18\),\(^19\) The present study was designed to determine (1) whether AT\(_2\)R stimulation is able to attenuate Ang II–induced cardiomyocyte hypertrophy and interstitial hyperplasia in TG mice and (2) whether the myocardial kinin/NO system is involved in AT\(_2\)R-mediated cardiac action.

Methods

Animals

TG mice (C57BL/6) that overexpressed the AT\(_2\)R selectively in cardiomyocytes by an \(\alpha\)-myosin heavy chain promoter were developed in Kansai Medical School.\(^18\),\(^19\) Homozygous littermates and

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wild-type (WT) littermates were sent to the Animal Research Center of Hiroshima University School of Medicine and maintained on a regular mouse chow and tap water. Two lines of transgenic mice, TG778 and TG 788, were used in the study. We previously demonstrated that phenotypes observed in TG778 and TG788 were mediated through overexpression of the transgene by blocking the AT$_R$ with PD123319, an AT$_R$ antagonist.¹⁸

Male TG mice and WT mice of 8 to 12 weeks of age were used in the study. Expressions of AT$_R$ gene and protein, with no change in the AT$_1$ receptor (AT$_1$-R), were confirmed by Northern blotting and ligand binding experiments using [¹²⁵]I-Sar$^1$Ile$^8$-Ang II as a ligand.¹⁸ This study was performed in accordance with the guidelines for animal experiments of Hiroshima University School of Medicine.

**Experimental Groups**

TG mice and WT mice were divided into 6 groups per strain and treated for a period of 14 days with (1) normal saline as a vehicle, (2) Ang II (1.4 mg/kg per day), (3) HOE140, a bradykinin B$_2$ receptor antagonist (400 µg/kg per day), (4) Ang II plus HOE140, (5) L-NAME, an NO synthase inhibitor, or (6) Ang II plus L-NAME. Ang II and HOE140 were subcutaneously infused through osmotic minipumps (Alzet model 2002, Alza Corp). L-NAME was given in the drinking water (0.1 mg/mL). This dose of L-NAME was chosen not to affect blood pressure in preliminary experiments. We tested 3 doses of L-NAME at 0, 0.1, 0.3, and 3 mg/mL in drinking water. Blood pressure after 2-week treatment was 92 ± 8, 95.8 ± 33, 115 ± 21, and 125 ± 30 mm Hg, respectively (n = 4 each). Conscious systolic blood pressure and heart rate were monitored before and during drug treatments by the tail-cuff method (BP98A, Softron).²,¹⁹

**Histological Analysis and Hydroxyproline Assay**

On day 14 of treatment, all mice were killed and hearts were excised. The left ventricle was immersion-fixed in 10% buffered formalin, and paraffin sections (1 to 2 µm) were cut. The myocyte cross-sectional area and myocardial fibrosis were quantitatively analyzed with NIH Image 1.61 software (National Institutes of Health Service Branch) in digitalized microscopic images as previously described.² For measurement of the cross-sectional area, 100 cells (per animal) from the left ventricular lateral-mid free wall (including epicardial and endocardial portions) were randomly chosen and analyzed.

Myocardial fibrosis in the tissue sections was quantitatively analyzed by morphometry in 2 ways: (1) focusing on the perivascular fibrosis and (2) focusing on the total fibroses including those in both perivascular areas and myocardial interstitial spaces. (1) The perivascular fibrosis of arteries was evaluated in short-axis images of intramuscular arteries and arterioles (at least 10 per animal) in Masson’s trichrome–stained sections. The area occupied by the artery (A) and the area of fibrosis surrounding the artery (B) were traced and calculated. The perivascular fibrosis index was defined as B/A. (2) The collagen both in myocardial interstitial spaces and perivascular areas was visualized by Sirius red staining and polarization microscopy, then quantified as previously described.¹,²⁰ The whole areas of the sections were scanned at ×200. The images were then digitalized and transformed into binary images, and the areas occupied by collagen were calculated by an automatic area-quantification program in NIH Image. The total interstitial fibrosis index was defined as the sum of the total area of collagen in the entire visual field divided by the sum of all connective tissue area plus the myocardial area in the entire visual field.²¹

As well as the morphometric analysis, the total collagen volume fraction was also determined by hydroxyproline content of the left ventricle by the use of a standard method.²² The lower half of each left ventricle was subjected to analysis.

**Northern Blot Analysis of Atrial Natriuretic Peptide and Reverse Transcriptase–Polymerase Chain Reaction for Transforming Growth Factor-$

Total RNA was extracted from the left ventricles of the mice on day 14 of treatment. Northern blot analysis of atrial natriuretic peptide (ANP) was performed as previously described by using full-length cDNA for mouse ANP. Reverse transcriptase–polymerase chain reaction (RT-PCR) for transforming growth factor-$

**Immunohistochemical Analysis**

Immunohistochemistry for bradykinin B$_2$ receptors (B$_2$R) and endothelial NO synthase (eNOS) was performed by using the formalin-fixed paraffin sections (2 µm) and unfixed frozen sections (6 µm), respectively.²,¹⁵,²³ For B$_2$R, sections were incubated for 1 hour at room temperature with a 1:100 dilution of monoclonal anti-B$_2$R antibody (B40820, Transduction Laboratory). For eNOS, the sections were incubated overnight at 4°C with a 1:1000 dilution of a polyclonal rabbit anti-eNOS antiserum (N30030, Transduction Laboratory). Signals for the B$_2$R and eNOS were visualized by the avidin-biotin immunoperoxidase method (Vectastain Vector M.O.M detection kit for B$_2$R and ABC Elite Kit for eNOS), with diamobenzidine used as substrate.

**Western Analysis**

Protein expressions of NO synthases (eNOS and inducible [i]NOS) and B$_2$-R in cardiac tissue were analyzed by Western blotting as described previously.²,¹⁵ Proteins were solubilized with 1% Triton X-100, separated by SDS-PAGE, transferred onto a PVDF membrane, and probed with anti-eNOS (N30030), anti-iNOS (N32030, Transduction Laboratory), or anti-B$_2$R antibody (B40820). The protein concentration was measured by the bicinchoninic acid method as described previously.²

**Receptor Assay**

Membrane fractions were prepared from the left ventricles of TG and WT mice treated with saline or Ang II for 14 days (n = 5 in each group). Receptor assays were performed as previously described by using [¹²⁵]I-[Sar$^1$Ile$^8$]-Ang II as a ligand. AT$_1$ and AT$_2$ densities (Bmax) were calculated as previously described on the basis of inhibition by CGP42112A and losartan, respectively.

**Measurement of Kininogenase Activity**

Kininogenase activity in heart homogenates was determined as an index of tissue kinin release as previously described.¹⁵ Components of the kallikrein-kinin system are identified in the heart.²⁴ Samples from heart homogenates were incubated with bovine kininogen (2000 ng, Seikagaku Kogyo) for 30 minutes at 37°C. The amount of kinins generated during the incubation was measured by radioimmunoassay. The kininogenase activity is expressed as the amount of kinins generated per milligram of protein per minute of incubation with kinogen.

**Statistical Analysis**

All data are expressed as mean ± SEM. Data for experimental groups were compared by ANOVA with Scheffé’s test for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

**Results**

The study was performed with 2 lines of TG mice (TG778 and TG788).¹⁸ We present the results of experiments in TG line 778, which showed the highest expression level of AT$_2$R in the heart. The ligand binding experiment suggested that the expression level of AT$_2$R protein in the ventricles of TG line 788 was 82.2% of that of TG line 778. The results obtained from TG788 were essentially comparable with TG778 line. The gene dose-response relation was unclear probably because the AT$_2$R expression level was so close.
Myocyte Hypertrophy

sectional area. Although left ventricular weight or on cross-sectional area (Figures 1A and 1B) in both TG and WT mice. However, the extent of the increases in these values was not different in the 2 strains. Treatment with HOE140 or L-NAME had no significant effect on the left ventricular weight or on cross-sectional area. Although left ventricular–to–body weight (LV/BW) ratios were larger in mice treated with Ang II+HOE140 or L-NAME than in mice treated with Ang II alone (Table 1), these larger ratios were due to a decrease in body weight caused by HOE140 or L-NAME treatment. Considering the fact that body weights in all groups were similar before treatments, absolute LV weights and cross-sectional areas rather than LV/BW ratios were thought to be better estimations for cardiomyocyte hypertrophy, suggesting that AT2-R overexpression did not affect cardiomyocyte hypertrophy in this experimental setting.

Left ventricular ANP mRNA levels were upregulated by Ang II to similar extents in TG and WT mice (Figure 2). Consistent with the results of absolute LV weights and cross-sectional areas (Table 1), the induction of ANP mRNA in TG mice was not significantly different from that in WT mice (Figure 2).

Effects of Cardiac Overexpression of the AT2-R on Myocyte Hypertrophy

Ang II infusion for 14 days significantly increased the left ventricular weight (Table 1) and the myocyte cross-sectional area (Figures 1A and 1B) in both TG and WT mice. However, the extent of the increases in these values was not different in the 2 strains. Treatment with HOE140 or L-NAME had no significant effect on the left ventricular weight or on cross-sectional area. Although left ventricular–to–body weight (LV/BW) ratios were larger in mice treated with Ang II+HOE140 or L-NAME than in mice treated with Ang II alone (Table 1), these larger ratios were due to a decrease in

Table 1. Effects of Ang II, HOE140, and L-NAME on Body Weight, Blood Pressure, Heart Rate, and Right and Left Ventricular Weights

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vehicle</th>
<th>Ang II</th>
<th>HOE</th>
<th>HOE+Ang II</th>
<th>L-NAME</th>
<th>L-NAME+Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transgenic mice, n</td>
<td>20</td>
<td>20</td>
<td>8</td>
<td>15</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>26.2±0.4</td>
<td>26.3±0.3</td>
<td>26.0±0.5</td>
<td>24.0±0.8*</td>
<td>26.9±0.7</td>
<td>24.6±0.6*</td>
</tr>
<tr>
<td>Right ventricular weight, mg</td>
<td>22.3±1.5</td>
<td>20.3±2.9</td>
<td>20.0±0.6</td>
<td>20.3±2.9</td>
<td>18.6±1.5</td>
<td>21.6±1.9</td>
</tr>
<tr>
<td>Left ventricular weight, mg</td>
<td>106.6±2.4</td>
<td>128.8±3.8**</td>
<td>116.7±2.3</td>
<td>124.1±4.5***</td>
<td>102.8±3.5</td>
<td>128±4.1***</td>
</tr>
<tr>
<td>Left ventricular/body weight rate, mg/g</td>
<td>4.08±0.07</td>
<td>4.94±0.15***</td>
<td>4.29±0.09</td>
<td>5.45±0.36*</td>
<td>4.01±0.11</td>
<td>5.25±0.17***</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>103.2±1.3</td>
<td>140.9±5.8*</td>
<td>104.8±2.6</td>
<td>141.4±7.0*</td>
<td>102.9±4.5</td>
<td>148.6±6.1*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>587±41</td>
<td>578±10</td>
<td>585±31</td>
<td>570±37</td>
<td>458±49</td>
<td>509±25</td>
</tr>
<tr>
<td>Wild-type mice, n</td>
<td>20</td>
<td>20</td>
<td>9</td>
<td>15</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>26.4±0.3</td>
<td>25.9±0.5</td>
<td>25.5±0.6</td>
<td>25.2±1.3</td>
<td>25.9±0.4</td>
<td>24.1±0.9*</td>
</tr>
<tr>
<td>Right ventricular weight, mg</td>
<td>21.5±2.0</td>
<td>21.2±2.9</td>
<td>19.0±3.2</td>
<td>20.5±1.8</td>
<td>21.0±1.6</td>
<td>19.4±2.5</td>
</tr>
<tr>
<td>Left ventricular weight, mg</td>
<td>107.1±1.4</td>
<td>121.7±3.3**</td>
<td>111.3±4.4</td>
<td>123.9±4.1***</td>
<td>107.6±2.1</td>
<td>121.4±5.6***</td>
</tr>
<tr>
<td>Left ventricular/body weight rate, mg/g</td>
<td>4.09±0.05</td>
<td>4.82±0.14***</td>
<td>4.29±0.19</td>
<td>5.12±0.21***</td>
<td>4.22±0.10</td>
<td>5.29±0.23***</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>98.6±1.5</td>
<td>141±4.3*</td>
<td>91.8±6.1</td>
<td>147.8±4.9*</td>
<td>101.4±2.9</td>
<td>140.9±7.6*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>549±22</td>
<td>548±28</td>
<td>491±17</td>
<td>625±30</td>
<td>400±26</td>
<td>505±67</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.05, **P<0.01 vs vehicle; †P<0.05, ††P<0.05 vs HOE; †††P<0.05 vs L-NAME; §§P<0.05 vs Ang II in the same strain.
nor the total fibrosis indexes were significantly different in TG and WT mice treated with Ang II plus HOE140 or L-NAME. Similarly, when treated with Ang II plus PD123319, an AT2R-specific antagonist, both TG and WT mice showed comparable perivascular fibrosis indexes (0.81±0.3 versus 0.78±0.5, n=3) and total fibrosis indexes (3.4±0.5 versus 3.6±0.4, n=3). The suppression of Ang II–induced fibrosis in TG mice was more readily detected in perivascular areas than in myocardial interstitial spaces. The reduction in the perivascular fibrosis index in Ang II–treated TG mice (62% compared with that in Ang II–treated WT mice) was larger than that in the total fibrosis index (46%), indicating that the antifibrotic effect of AT2-R overexpression may be more prominent in perivascular areas than in myocardial interstitial spaces.

Consistent with the results of the perivascular fibrosis index and total fibrosis index, the LV hydroxyproline content was increased by Ang II both in TG and WT mice, whereas the extent was significantly less in TG mice (Figure 4). Coadministration of HOE140 or L-NAME in addition to Ang II abolished the difference in TG and WT mice.

**TGF-β1 mRNA Expression**

To determine whether suppression of fibrosis is mediated by change in TGF-β1, we measured TGF-β1 by RT-PCR. As shown in Figure 5, TGF-β1 mRNA was significantly in-
increased by treatment with Ang II, Ang II+HOE140, or Ang II+L-NAME, the extent of which was not significantly different in TG and WT mice. Treatment with HOE140 or L-NAME alone had no significant effects.

Kininogenase Activity
We next investigated whether cardiac AT2-R stimulation activates the kinin-kallikrein system. There was no significant difference between kininogenase activities in TG and WT mice treated with the vehicle. In TG mice but not WT mice, Ang II infusion significantly increased (≈2.6 fold, P<0.01) the kininogenase activity (Table 2).

Localizations of B2R and NOS
Since cotreatment of HOE140 or L-NAME with Ang II abolished the AT2-R–mediated action on perivascular fibrosis, we examined the localization of B2R (Figure 6) and expression and localization of NO synthases, including eNOS (Figure 7) and iNOS, in the hearts of TG and WT mice.
B2Rs were most abundantly expressed in the vascular endothelium and to a lesser extent in fibroblasts (Figure 6A). No positive signals were detected in cardiomyocytes. eNOS was localized to the vascular endothelium (Figure 7A) but was not present in the vascular smooth muscle layer, fibroblasts, or cardiomyocytes. Taken together, both B2R and eNOS were expressed in the endothelium, whereas only B2R was present in fibroblasts, and neither was present in cardiomyocytes. The localizations and expression levels of B2R and eNOS were similar in TG and WT mice and not affected by any treatments. The localization of B2R and eNOS in the vascular endothelium but not in fibroblasts suggests that bradykinin released by AT2R stimulation is involved in inhibition of perivascular fibrosis through activation of the B2R/NO system in the vascular endothelium.

Western blot analyses (Figure 6B and Figure 7B) and densitometric analyses (n=4, Figure 6C and Figure 7C) demonstrated that similar amounts of B2Rs and eNOS proteins were expressed in the hearts of TG and WT mice and that Ang II or any other drug treatment did not change the expression levels of B2Rs and eNOS. iNOS protein was not detected either by Western blotting or by immunohistochemistry under any conditions (data not shown).

Discussion
This study was designed to determine whether cardiomyocyte-specific overexpression of the AT2-R modulates myocyte growth and interstitial fibrosis. The major finding is that perivascular fibrosis was inhibited by stimulation of myocyte AT2-Rs in a bradykinin/NO-dependent manner. It is thought that bradykinin released from cardiomyocytes through AT2-R signaling activates endothelial B2Rs, leading to activation of eNOS followed by NO-dependent inhibition of fibrosis in perivascular fibroblasts. Recently, 2 groups have described the effects of targeted disruption of the AT2-R on pressure overload-induced cardiac hypertrophy in mice. Akishita et al demonstrated that disruption of this receptor subtype enhanced coronary vascular remodeling and perivas-

Figure 4. Bar graph shows hydroxyproline content in LV as an index for LV total collagen volume fractions. Results are expressed as mean±SEM. n=6 to 7.

\*P<0.05 vs Ang II.

Figure 5. RT-PCR for TGFβ1 in left ventricles of AT2-TG and WT control mice. Top, Agarose gel electrophoresis of RT-PCR products shows bands for TGFβ1 and 18S ribosome RNA. Bottom, Bar graph shows results of densitometric analysis of intensity of TGFβ1 relative to that of 18S ribosome RNA. n=4.

\*P<0.05 vs same strain of mice treated with Ang II.
cular fibrosis but had no effect on cardiomyocyte hypertrophy. In contrast, Senbonmatsu et al.\(^\text{17}\) reported that targeted deletion of the AT\(_2\)R prevents LV hypertrophy as well as interstitial fibrosis. The reason for these conflicting results is not known. Akishita et al.\(^\text{16}\) and Senbonmatsu et al.\(^\text{17}\) established AT\(_2\)R-null mice in different strains, for example, C57/BL and FVB strains, respectively. Our cardiomyocyte-specific AT\(_2\)R TG mice were originated from C57/BL strain, whereas our result was consistent with the observation by Akishita et al.\(^\text{16}\) in FVB strain.

In the present study, the antifibrotic effect of AT\(_2\)R overexpression was much more prominent in the perivascular area (Figures 3A and 3B) than in the interstitial space (Figures 3C and 3D). The relatively short-term infusion of Ang II used in the study resulted in development of fibrosis mainly in the perivascular area but not in the myocardial interstitial space, findings that are consistent with an early feature of Ang II–induced myocardial fibrosis.\(^\text{1}\) Therefore, it is thought that differences between the amounts of interstitial fibrosis in the 2 strains, if any, may have been difficult to detect. The antifibrotic effect of AT\(_2\)R overexpression was abolished by treatment with HOE140 and L-NAME, suggesting that bradykinin/NO plays a critical role in the inhibition of fibrosis in Ang II–treated TG mice. In our experimental conditions, both B\(_2\)R (Figure 6) and eNOS (Figure 7) were mainly localized in the vascular endothelium and iNOS was not detected. These findings suggest that the perivascular area might be the main site of action for bradykinin/NO to inhibit fibrosis in TG mice.

It has been shown that AT\(_2\)R has an antihypertrophic effect in cultured neonatal rat ventricular myocytes.\(^\text{3,5}\) In the present study, AT\(_2\)R overexpression in cardiomyocytes had no effect on Ang II–induced cardiomyocyte growth. However, we cannot conclude that AT\(_2\)R is not involved in the mechanism of cardiomyocyte hypertrophy for the following reasons. One reason is that we cannot exclude the possibility that the expression level of AT\(_2\)R might have been too low relative to that of AT\(_1\)R.\(^\text{25}\) The level of cardiac AT\(_2\)-R in our TG mice was \(/30\%\) of that of AT\(_1\)-R, whereas studies demonstrating an antihypertrophic effect of AT\(_2\)-R on cardiomyocytes\(^\text{3,5}\) have been performed on cultured cardiomyocytes that express nearly equal levels of AT\(_2\)-R and AT\(_1\)-R.\(^\text{3}\) In patients with dilated cardiomyopathy, AT\(_2\)R in the heart was upregulated by 3- to 4-fold.\(^\text{6}\) The second reason is that we infused a

### Table 2. AT\(_2\)-R-Mediated Increase of Cardiac Kininogenase Activity

<table>
<thead>
<tr>
<th>Strain</th>
<th>Vehicle (n=8)</th>
<th>Ang II (n=8)</th>
<th>Hoe 140 (n=4)</th>
<th>Hoe 140+Ang II (n=4)</th>
<th>L-NAME (n=4)</th>
<th>L-NAME+Ang II (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transgenic</td>
<td>178±11</td>
<td>447±18*</td>
<td>177±15</td>
<td>441±15*</td>
<td>188±14</td>
<td>425±14*</td>
</tr>
<tr>
<td>Wild-type</td>
<td>166±13</td>
<td>175±15</td>
<td>173±10</td>
<td>165±13</td>
<td>169±10</td>
<td>170±10</td>
</tr>
</tbody>
</table>

Ang II (1.4 mg/kg per day) was chronically infused into WT or TG mice for 14 days, and the left ventricles were isolated. Kininogenase activities in homogenates of apical parts were determined by measuring the kinin generated from added kininogen as described in Methods. Values denote kininogenase activity (pg/mg protein/min) and are expressed as mean±SEM.

\(*P<0.001\) vs vehicle.

![Figure 6.](hyper.ahajournals.org)
pressor dose of Ang II for 2 weeks, which caused a significant increase in blood pressure and might have obscured any direct growth/antigrowth effects of Ang II on cardiomyocytes.

Although AT2R was not detected in WT mice by ligand binding analysis, cardiac AT2Rs have been identified by immunohistochemistry in mice and in rats. It has been reported that AT2Rs were present in interstitial fibroblasts or in the perivascular area, inhibiting fibrosis and vascular remodeling. Wang et al have demonstrated in the rat that AT2R is expressed also in cardiomyocytes. In the present study, AT2R overexpressed in cardiomyocytes inhibited interstitial fibrosis. Taken together, AT2Rs are normally expressed in the heart, and this receptor subtype may function as an antifibrotic factor regardless of its localization.

Perspectives
We demonstrated that stimulation of myocardial AT2Rs inhibits perivascular fibrosis through a bradykinin/NO-dependent mechanism. The results of this study have important clinical implications. Administration of an AT1-receptor antagonist causes elevation in the plasma level of Ang II, which will specifically bind to AT1Rs in the heart and may serve as an AT1R agonist. The proportion of AT1Rs relative to total Ang II binding capacity in the human heart is much higher than that observed in animals. The results of our study suggest that the beneficial effect of an AT1 receptor antagonist on fibrosis in humans may be, at least in part, mediated by the AT2R. Elucidation of the beneficial role of the AT2R in the human heart would contribute to the establishment of more sophisticated methods of treatment for human heart diseases.

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