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

Satoshi Kurisu, Ryozo 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 AT2 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 AT2 receptor relative to AT1 receptor in transgenic mice was 22% to 37%. Ang II caused similar elevations in systolic blood pressure (by ≈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 B2 receptor antagonist, or L-NAME, an inhibitor of NO synthase. Cardiac kininogenase activity was markedly increased (∼2.6-fold, P<0.001) after Ang II infusion in transgenic mice but not in wild-type mice. Immunohistochemistry indicated that both bradykinin B2 receptors and endothelial NO synthase were expressed in the vascular endothelium, whereas only B2 receptors were present in fibroblasts. These results suggest that stimulation of AT1 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, AT1 and AT2, are expressed in the heart.2 The AT1 receptor (AT1R) has been implicated in suppression of myocardial hypertrophy,3–5 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 AT2R in cardiac diseases in vivo. Previous studies have suggested possible involvement of the bradykinin/nitric oxide (NO)/cGMP system in AT1R-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 AT1R 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 AT1R to cardiac bradykinin synthesis and activation of the NO system. It has also not been determined whether stimulation of the AT2R and/or bradykinin has direct effects on myocyte hypertrophy and interstitial hyperplasia. Studies conducted in 2 laboratories6,16,17 with AT2R-null mice showed conflicting results, the reason for which remains to be determined.

We have recently developed transgenic (TG) mice overexpressing the AT2R selectively in cardiomyocytes.18,19 The present study was designed to determine (1) whether AT2R 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 AT2R-mediated cardiac action.

Methods

Animals
TG mice (C57BL/6) that overexpressed the AT2R selectively in cardiomyocytes by an α-mysin heavy chain promoter were developed in Kansai Medical School.18,19 Homozygous littermates and

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From the First Department of Internal Medicine (S.K., T.I., H.S., H. Matsuura, K.C.) and Clinical Laboratory Medicine (R.O., T.O., M.K.), Second Department of Physiology (Y.T.), Hiroshima University School of Medicine, Hiroshima, Japan; and Internal Medicine II, Kansai Medical University (O.I., K.A., H. Matsubara), Osaka, Japan.
Correspondence to Ryoji Ozono, MD, Department of Clinical Laboratory Medicine, Hiroshima University Faculty of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima, Japan 734-8551. E-mail ozono@hiroshima-u.ac.jp
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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 AT₂R with PD123319, an AT₂R antagonist.¹⁸

Male TG mice and WT mice of 8 to 12 weeks of age were used in the study. Expression of AT₂R gene and protein, with no change in the AT₁ receptor (AT₁-R), were confirmed by Northern blotting and ligand binding experiments by using [¹²⁵I]-Sar¹Ile⁸-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 B₂ 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 0.1, 0.3, and 3 mg/mL in drinking water. Blood pressure after 2-week treatment was 92±8, 95±8.33, 115±2.1, 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 fibrosis including those in both perivascular areas and myocardial interstitial spaces. (1) The perivascular fibrosis of arteries was evaluated in short-axis images of intramural 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 heart. The ligand binding experiment suggested that the expression level of AT₂ R protein in the ventricles of TG line 778, which showed the highest expression level of AT₂ R in the heart, was 82.2% of that of TG line 778. The results obtained from TG788 were essentially comparable with TG778 line. We present the results of experiments in TG mice lines 778 and 788.¹⁸

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 kininogen.

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 AT₁ R in the heart. The ligand binding experiment suggested that the expression level of AT₁ R protein in the ventricles of TG line 778 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 AT₁ R expression level was so close.
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>
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<tr>
<td>Transgenic mice, n</td>
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<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>24.8±0.6*</td>
<td>26.9±0.7</td>
<td>24.8±0.6*</td>
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<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>
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<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>108.2±3.5</td>
<td>128±4.1***</td>
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<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>
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<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>141.8±7.0*</td>
<td>102.9±4.5</td>
<td>148.6±6.1*</td>
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<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>
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<td>Wild-type mice, n</td>
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<td>10</td>
<td>10</td>
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<tr>
<td>Body weight, g</td>
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<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>
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<tr>
<td>Left ventricular, 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>
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<tr>
<td>Left ventricular/body weight rate, mg/g</td>
<td>4.09±0.05</td>
<td>4.82±0.14***</td>
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<td>5.12±0.21***</td>
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<td>Systolic BP, mm Hg</td>
<td>98.6±1.5</td>
<td>141±4.3*</td>
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<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>
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</table>

Values are mean±SEM.  
*P<0.05, **P<0.01 vs vehicle; †P<0.05, ††P<0.01 vs HOE; ‡P<0.05, ‡‡P<0.01 vs L-NAME; §P<0.05 vs Ang II in the same strain.

Changes in Body Weight, Blood Pressure, and Densities of AT1-R and AT2-R After Ang II Treatment

There were no significant differences in systolic blood pressure, heart rate, or body weight among the 12 groups before treatment. On day 14, the body weights of mice given Ang II+HOE140 or Ang II+L-NAME were lower than those of the vehicle-treated mice, but there was no significant difference between the body weights of TG and WT mice receiving the same treatments (Table 1). Systolic blood pressures of TG and WT mice treated with Ang II, Ang II+HOE140, or Ang II+L-NAME were elevated to a similar extent (by 45 mm Hg) (Table 1).

The Ang II infusion did not significantly change the total binding of Ang II in either the TG or WT mice (total binding in mice treated with the vehicle versus those treated with Ang II: 31±5 versus 32±4 fmol/mg in TG mice and 26±6 versus 28±4 fmol/mg protein in WT mice, respectively). The ratio of AT1-R versus AT2-R also was not significantly altered by Ang II treatment: The ratios were 73:27% in TG mice treated with the vehicle and 100:0% in WT mice treated with the vehicle, 82:18% in TG mice treated with Ang II, and 100:0% in WT mice treated with Ang II. Neither change in the amount of AT1-R or AT2-R after Ang II infusion was statistically significant.

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 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 Fibrosis in Perivascular Areas and Myocardial Interstitial Spaces

Administration of Ang II caused fibrotic changes mainly in the perivascular area and to a lesser extent in the myocardial interstitial space of the heart both in TG and WT mice. Figure 3A shows short-axis images of intramuscular arteries with perivascular fibrosis that were stained with Masson’s trichrome, and Figure 3B is the results of the quantitative morphometry. Figure 3C shows interstitial collagen deposition around relatively smaller arteries that were visualized with Sirius red staining and polarization, and Figure 3D is the result of the quantitative analysis. Fibrosis was also observed in myocardial interstitial spaces in mice treated with Ang II, but the extent of the myocardial interstitial fibrosis was much less than that of perivascular fibrosis. Collagen weave did not appear to be different between the strains.

Notably, as revealed by the perivascular index (Figure 3B) and total interstitial fibrosis index (Figure 3D), the Ang II–induced perivascular fibrosis was significantly suppressed in TG mice compared with that in WT mice, and coadministration of HOE140 or L-NAME with Ang II abolished the difference between the amounts of Ang II–induced fibrosis in TG and WT mice. Neither the perivascular fibrosis indexes...
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-

Figure 1. Left ventricular myocyte cross-sectional areas in AT2-TG and WT control mice. A, Cross sections of cardiomycocytes stained with Masson’s trichrome. B, Bar graph shows quantitative analysis of cross-sectional area. Results are expressed as mean±SEM. n=8. **P<0.05 vs all 3 groups receiving vehicle, HOE140, and L-NAME in the same strain.

Figure 2. Northern blot analysis of ANP in AT2-TG and WT control mice. A, Autoradiogram of ANP gene expression in ventricles. B, Bar graph shows results of densitometric analysis of ANP mRNA. Results are expressed as mean±SEM, n=5. **P<0.01 vs all 3 groups receiving vehicle, HOE140, and L-NAME in the same strain.
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.

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Figure 3. Assessment of cardiac fibrosis in AT2-TG mice and WT mice. A, Short-axis images of intramuscular arteries with perivascular fibrosis stained with Masson’s trichrome. Scale bar indicates 50 \( \mu \)m. B, Bar graph shows quantified perivascular fibrosis index (n=8). Note that Ang II–induced perivascular fibrosis was significantly less in TG mice than in WT mice, but this difference was abolished by coadministration of HOE140 or L-NAME with Ang II. C, Collagen deposition in the perivascular area (arrows) and myocardial interstitial space visualized with Sirius red staining and polarization. Smaller arteries than in A are shown. Arrowheads indicate epicardial edge. Ang II–induced perivascular fibrosis was again inhibited in TG mice. Myocardial interstitial fibrosis was generally mild regardless of the treatments and mouse strain. Scale bar indicates 50 \( \mu \)m. D, Bar graph showing myocardial total fibrosis index (n=8). Results in B and D are expressed as mean±SEM. **\( P<0.01 \) vs all 3 groups receiving vehicle, HOE140, and L-NAME, †\( P<0.01 \) vs AT2-TG mice receiving Ang II+HOE140 and Ang II+L-NAME, §\( P<0.01 \) vs WT mice receiving Ang II alone.
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 AT1-Rs in a bradykinin/NO-dependent manner. It is thought that bradykinin released from cardiomyocytes through AT1R 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 AT2R on pressure overload-induced cardiac hypertrophy in mice. Akishita et al demonstrated that disruption of this receptor subtype enhanced coronary vascular remodeling and perivas-
cular fibrosis but had no effect on cardiomyocyte hypertrophy. In contrast, Senbonmatsu et al.\(^7\) reported that targeted deletion of the AT2R prevents LV hypertrophy as well as interstitial fibrosis. The reason for these conflicting results is not known. Akishita et al.\(^6\) and Senbonmatsu et al.\(^7\) established AT2R-null mice in different strains, for example, C57/BL and FVB strains, respectively. Our cardiomyocyte-specific AT2R TG mice were originated from C57/BL strain, whereas our result was consistent with the observation by Akishita et al.\(^6\) in FVB strain.

In the present study, the antifibrotic effect of AT2R 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.\(^1\) 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 AT2R 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 B2R (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 AT2R has an antihypertrophic effect in cultured neonatal rat ventricular myocytes.\(^3,5\) In the present study, AT2R overexpression in cardiomyocytes had no effect on Ang II–induced cardiomyocyte growth. However, we cannot conclude that AT2R 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 AT2R might have been too low relative to that of AT1R.\(^2,5\) The level of cardiac AT2R in our TG mice was 30% of that of AT1R, whereas studies demonstrating an antihypertrophic effect of AT2R on cardiomyocytes\(^3,5\) have been performed on cultured cardiomyocytes that express nearly equal levels of AT2R and AT1R.\(^3\) In patients with dilated cardiomyopathy, AT2R in the heart was upregulated by 3- to 4-fold.\(^6\) The second reason is that we infused a

![Table 2](https://example.com/table2.png)

**Table 2. AT2R-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>
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<tr>
<td>Transgenic</td>
<td>178±11</td>
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</table>

*P<0.001 vs vehicle.

![Figure 6](https://example.com/figure6.png)

**Figure 6.** A. Immunohistochemical localization of B2R in left ventricles of TG and WT mice. B2Rs were localized in vascular endothelium (red arrow) and to a lesser extent in fibroblasts (black arrow). There were no detectable signals in cardiomyocytes. Localization and expression levels of B2Rs were similar in TG and WT mice. B. Western blot analysis of B2R protein. B2R protein expression patterns did not differ in TG and WT and were not changed by drug treatments. The membrane was reprobed with actin as an internal standard. C. Bar graph shows results of densitometric analysis of B2R expression (n=4, each group).
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 AT$_2$R was not detected in WT mice by ligand binding analysis, cardiac AT$_2$Rs have been identified by immunohistochemistry in mice$^{16}$ and in rats.$^{2,26}$ It has been reported that AT$_2$Rs were present in interstitial fibroblasts$^{6,27}$ or in the perivascular area,$^{16}$ inhibiting fibrosis and vascular remodeling. Wang et al.$^{26}$ have demonstrated in the rat that AT$_2$R is expressed also in cardiomyocytes. In the present study, AT$_2$R overexpressed in cardiomyocytes inhibited interstitial fibrosis. Taken together, AT$_2$Rs 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 AT$_2$Rs inhibits perivascular fibrosis through a bradykinin/NO-dependent mechanism. The results of this study have important clinical implications. Administration of an AT$_1$-receptor antagonist causes elevation in the plasma level of Ang II,$^{28}$ which will specifically bind to AT$_2$Rs in the heart and may serve as an AT$_2$R agonist. The proportion of AT$_2$Rs relative to total Ang II binding capacity in the human heart is much higher than that observed in animals.$^{6}$ The results of our study suggest that the beneficial effect of an AT$_1$ receptor antagonist on fibrosis in humans may be, at least in part, mediated by the AT$_2$R. Elucidation of the beneficial role of the AT$_2$R in the human heart would contribute to the establishment of more sophisticated methods of treatment for human heart diseases.

**Acknowledgments**

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References


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