Pressure Overload Induces Cardiac Hypertrophy in Angiotensin II Type 1A Receptor Knockout Mice

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Background—Many studies have suggested that the renin-angiotensin system plays an important role in the development of pressure overload–induced cardiac hypertrophy. Moreover, it has been reported that pressure overload–induced cardiac hypertrophy is completely prevented by ACE inhibitors in vivo and that the stored angiotensin II (Ang II) is released from cardiac myocytes in response to mechanical stretch and induces cardiomyocyte hypertrophy through the Ang II type 1 receptor (AT1) in vitro. These results suggest that the AT1-mediated signaling is critical for the development of mechanical stress–induced cardiac hypertrophy.

Methods and Results—To determine whether AT1-mediated signaling is indispensable for the development of pressure overload–induced cardiac hypertrophy, pressure overload was produced by constricting the abdominal aorta of AT1A knockout (KO) mice. Quantitative reverse transcriptase–polymerase chain reaction revealed that the cardiac AT1 (probably AT1B) mRNA levels in AT1A KO mice were 10% of those of wild-type (WT) mice and were not affected by pressure overload. Chronic treatment with suppressor doses of Ang II increased left ventricular mass in WT mice but not in KO mice. Pressure overload, however, fully induced cardiac hypertrophy in KO as well as WT mice. There were no significant differences between WT and KO mice in expression levels of fetal-type cardiac genes, in the left ventricular wall thickness and systolic function as revealed by the transthoracic echocardiogram, or in the histological changes such as myocyte hypertrophy and fibrosis.

Conclusions—AT1-mediated Ang II signaling is not essential for the development of pressure overload–induced cardiac hypertrophy.

Key Words: hypertrophy ■ pressure ■ angiotensin ■ genes

A growing body of evidence suggests that Ang II plays an important role in the development of cardiac hypertrophy.1,2 ACE inhibitors or Ang II receptor antagonists induce the regression and prevent the development of cardiac hypertrophy both in experimental animal models3-7 and in hypertensive patients.8-10 Many studies have demonstrated that hemodynamic overload activates the tissue RAS in the heart.11-16 mRNA and/or protein levels of renin,11 ACE,12,13 angiotensinogen,5,11,14 and Ang II receptors15,16 have been reported to be increased in hypertrophied hearts. In addition to these in vivo studies, mechanisms by which mechanical stress induces cardiomyocyte hypertrophy have also been investigated in vitro with cultured cardiac myocytes. We have developed an in vitro system of stretching cultured cardiac myocytes seeded on deformable silicon dishes.17,18 Using this system, we demonstrated that mechanical stretch of cultured cardiac myocytes activates the phosphorylation cascade of protein kinases, induces the expression of immediate early genes and fetal-type genes, and increases the protein synthesis rate.17-21 Furthermore, we reported that Ang II plays a critical role in the mechanical stretch–induced cardiomyocyte hypertrophy.21 It was recently reported that Ang II is stored in the secretory granules of cardiac myocytes and that secretion is induced by mechanical stress.23 However, mechanical stretch–induced cardiomyocyte hypertrophy was not completely prevented by Ang II receptor antagonists,22 suggesting that there might be signaling pathways other than those provoked by Ang II. Indeed, endothelin-1 (ET-1) also plays a pivotal role in the development of mechanical stress–induced cardiomyocyte hypertrophy.24 Recent studies have also demonstrated that there are some differences between Ang II– and mechanical stress–induced signaling pathways in cardiac myocytes.22,25,26 Therefore, it is questionable whether Ang II is indispensable for the development of mechanical stress–induced cardiac hypertrophy.

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The intracellular signals are evoked by Ang II through seven transmembrane Ang II receptors.27,28 At present, Ang II
receptors are divided into two major subtypes, AT₁ and AT₂ receptors, and AT₁ receptors are further subdivided into AT₁A and AT₁B receptors, which are the products of different genes. They is generally accepted from many studies using subtype-specific receptor antagonists that most of the well-known Ang II functions in the cardiovascular system are mediated by AT₁. Studies of gene targeting have clearly shown that AT₁-mediated Ang II signaling is essential for the maintenance of systemic blood pressure. In contrast, the physiological roles of AT₂ in the cardiovascular system were largely unknown. Recent studies of AT₂ KO mice, however, have revealed that AT₂ is also involved in the regulation of systemic blood pressure. These results suggest that genetically engineered mice are powerful tools to clarify the pathophysiological roles of the RAS in cardiovascular systems.

In the present study, to examine whether Ang II is essential for the development of pressure overload–induced cardiac hypertrophy, chronic pressure overload was produced in the heart of AT₁A KO mice. Chronic pressure overload induced cardiac hypertrophy in KO mice as well as in WT mice, although the administration of subpressor doses of Ang II does not induce cardiac hypertrophy in KO mice. Pressure overload fully induced all phenotypes observed in cardiac hypertrophy, such as cardiomyocyte hypertrophy, reprogramming of gene expression, and perivascular fibrosis in both kinds of mice. These results suggest that pressure overload can induce cardiac hypertrophy without the AT₁-mediated Ang II signaling pathways.

Methods

Animals
AT₁A KO mice (n = 10) and WT mice (n = 10), 18 weeks old, from the same genetic background were used in the present study. Mice were housed under climate-controlled conditions with a 12-hour light/dark cycle and were provided with standard food and water ad libitum. All protocols were approved by local institutional guidelines.

Development of Pressure Overload–Induced Cardiac Hypertrophy
Pressure overload was produced by constriction of the abdominal aorta as described previously. Briefly, mice were anesthetized by injection of sodium pentobarbital (30 mg/kg IP). The abdominal aorta was constricted at the suprarenal level with 7–0 nylon strings by ligation of the aorta with a blunted 29-gauge needle, which was pulled out thereafter. To monitor the hemodynamic effects of aortic constriction at 40 days after aortic banding, the left carotid artery was cannulated with stretched PE 50 tubing, which was tunneled under the skin and exteriorized posteriorly at the base of the neck. After the mice had completely recovered from anesthesia, blood pressure was measured in conscious mice under unrestrained conditions and was recorded continuously over a period of 60 minutes by a polygraph system (Nihon Koden Co). On the basis of the arterial blood pressure recordings during this period, a mean value for systolic pressure was calculated. After blood pressure was recorded, hearts were excised, weighed, and subjected to further analysis.

Quantitative RT-PCR Analysis
Total RNA was prepared from the hearts of mice by use of RNA STAT-60 (TEL-TEST “B,” Inc) followed by digestion with DNase (Takara Shuzo) to eliminate any contamination of genomic DNA. The RT-PCR analysis for AT₁ and AT₂ mRNA quantification was performed with the deletion-mutated cRNA as described previously. The amplification efficiencies of target and competitor transcripts are equal under optimal concentrations of competitor transcripts. The oligonucleotide primers used for RT-PCR analysis are as follows: for AT₁, 5'-GGATGCTGTTTCCACCGATCAGCATCAGC-3' and 5'-GGATGACGCCCAGCTGAATCAGCA-3', and for AT₂, 5'-TGTGGCACCAGCAGAAC-3' and 5'-GTGGGGCTCCAAACCATGGTCA-3'. The sequence of these primers is identical to that of murine Ang II receptor cDNA.

To verify that equal amounts of RNA were subjected to RT-PCR, GAPDH mRNA was also amplified with the following primers: 5'-GGATGCTGTTTCCACCGATCAGCATCAGC-3' and 5'-GGATGACGCCCAGCTGAATCAGCA-3'. Denaturing (94°C for 45 seconds), annealing (58°C for 1 minute), and extension (72°C for 1 minute) reactions were performed for 30 cycles. Because the primers used for the amplification of AT₁ correspond to common sequences between AT₁A and AT₁B, both AT₁A and AT₁B mRNAs were amplified. When PCR was performed without the step of RT, no PCR product was amplified, indicating that the product was not generated by the amplification of contaminated genomic DNA. The range of concentrations of sample RNA and internal control–deleted cRNA, as well as the number of amplification cycles, was selected from within the exponential phase. To determine the amount of mRNA, 5 μCi of [α-32P]dCTP was included in the PCR reaction mixtures, and the incorporated 32P activity was measured with a scintillation counter.

Chronic Administration of Subpressor Dose of Ang II
An osmotic minipump (model 2002, Alza Corp) was implanted subcutaneously into mice. Subpressor doses of Ang II (100 ng·kg⁻¹·min⁻¹) and 0.01 mol/L acetic acid in saline or saline alone were administered for 14 days, and arterial blood pressure was measured as described above. After blood pressure was recorded under unrestrained conditions, hearts were rapidly excised and weighed.

Northern Blot Analysis
Ten micrograms of total RNA was separated on a 1.2% agarose/formaldehyde gel and blotted onto Hybond-N membrane (Amersham Co). cDNA of ANP, BNP, and SERCAwere used as probes. Hybridizing bands were quantified with a FUJIX Bio-Imaging Analyzer BAS 2000 (Fuji Film Co).

Echocardiographic Analysis
Transsthoracic echocardiography was performed with HP Sonos 100 (Hewlett-Packard Co) with a 10-MHz imaging transducer as described previously. Mice were anesthetized with ketamine (10 mg/kg IP) and xylazine (15 mg/kg IP). After a good-quality two-dimensional image was obtained, M-mode images of the left ventricle were recorded. Intraventricular septum thickness, end-diastolic left ventricular internal diameter (EDD), end-systolic left ventricular internal diameter (ESD), and left ventricular posterior wall thickness were measured and analyzed.

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SELECTED ABBREVIATIONS AND ACRONYMS

Ang II = angiotensin II
ANP = atrial natriuretic peptide
AT₁ = angiotensin II type 1 receptor
AT₁A = angiotensin II type 1A receptor
AT₁B = angiotensin II type 1B receptor
AT₂ = angiotensin II type 2 receptor
BNP = brain natriuretic peptide
ET-1 = endothelin-1
KO = knockout
MHC = myosin heavy chain
PCR = polymerase chain reaction
RAS = renin-angiotensin system
RT = reverse transcriptase
SERCA = sarcoplasmic reticulum Ca²⁺-ATPase
WT = wild-type
cardiac hypertrophy.\(^1\)\(^5\) \(^6\) AT\(^1\) and AT\(^2\) mRNA levels in KO mice were significantly lower than those in WT mice (WT, 43 ± 7 mm Hg; KO, 92 ± 6 mm Hg; \(P < .001\)) (Fig 3A). Consistent with a previous report,\(^29\) unexpectedly, the heart weight/body weight ratio was markedly increased not only in WT mice but also in KO mice, and the degree of increase in this ratio was almost identical between WT and KO mice (WT, 29% increase; KO, 33% increase) (Fig 3B), indicating that pressure overload can increase left ventricular weight without the AT\(^1\)-mediated signaling.

## Results

### Quantitative RT-PCR Analysis of AT\(^1\) and AT\(^2\) mRNA in Murine Hearts

Because AT\(^1\) mRNA levels were too low to be detected by Northern blot analysis or RNase protection assay, we subjected mRNA levels of Ang II receptors to semiquantitative analysis by competitive RT-PCR methods as described previously.\(^15\)\(^16\)\(^34\) mRNA levels of cardiac AT\(^1\) gene in KO mice were <10% of those in WT mice (Fig 1A). Because the PCR products of AT\(^1\) in AT\(^1\) KO mice were completely digested by \(H\)incII (data not shown), this slight expression of AT\(^1\) observed in KO hearts should represent AT\(^1\) mRNA transcripts.\(^42\) In WT mice, cardiac AT\(^1\) mRNA levels were significantly higher in hypertrophied hearts than in control hearts (1.5-fold; \(P < .05\)), whereas no significant difference was observed in the amount of AT\(^2\) mRNA levels between control and hypertrophied hearts (Fig 1B), in good agreement with the previous study of murine hypertrophied hearts.\(^43\) In contrast, cardiac mRNA levels of both AT\(^1\) and AT\(^2\) were not affected by pressure overload in KO mice (Fig 1A and 1B).

### Effects of Subpressor Doses of Angiotensin II on Development of Cardiac Hypertrophy

AT\(^1\) and AT\(^1\) are 96% identical at amino acid levels and pharmacologically indistinguishable from each other.\(^28\) Because RT-PCR analysis has revealed that trace amounts of AT\(^1\) (probably AT\(^1\)) exist in the heart of KO mice, the possible involvement of AT\(^1\) in the development of cardiac hypertrophy was examined. For this purpose, we continuously administered a subpressor dose (100 ng · kg\(^{-1}\) · min\(^{-1}\)) of Ang II into WT and KO mice for 2 weeks. Systolic blood pressure (Fig 2A) and body weight (data not shown) were not changed by the administration of Ang II in any mice. The Ang II treatment, however, markedly increased the heart weight/body weight ratio in WT mice but not in KO mice.

The results strongly suggest that in KO hearts, Ang II does not evoke enough signals through AT\(^1\) to lead to the development of cardiac hypertrophy.

### Cardiac Hypertrophy by Abdominal Aortic Banding

Many laboratories have reported that cardiac hypertrophy is induced by constriction of the abdominal aorta through activation of the RAS.\(^5\)\(^7\) At 40 days after operation, blood pressure was still elevated in both WT and KO mice. The increase in systolic blood pressure was not significantly different between WT and KO mice (WT, 37 ± 7 mm Hg; KO, 43 ± 7 mm Hg), although the baseline blood pressure of KO mice was significantly lower than that of WT mice (WT, 127 ± 5 mm Hg; KO, 92 ± 6 mm Hg; \(P < .001\)) (Fig 3A), consistent with a previous report.\(^29\) Unexpectedly, the heart weight/body weight ratio was markedly increased not only in WT mice but also in KO mice, and the degree of increase in this ratio was almost identical between WT and KO mice (WT, 29% increase; KO, 33% increase) (Fig 3B), indicating that pressure overload can increase left ventricular weight without the AT\(^1\)-mediated signaling.

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**Figure 1.** Cardiac AT\(^1\) and AT\(^2\) mRNA levels before and 40 days after aortic banding. RT-PCR analysis was performed to evaluate mRNA levels of AT\(^1\) (A) and AT\(^2\) (B). Normalized values of AT\(^1\) and AT\(^2\) in WT heart before aortic banding are arbitrarily expressed as 100% (right). RT-PCR was performed with primers specific for murine AT\(^1\) and AT\(^2\) in reaction mixture containing [\(\alpha\)-\(^32\)P]dCTP. Amplified DNA was electrophoresed in 1% agarose gel and stained with ethidium bromide (left). AT\(^1\) and AT\(^2\) mRNA levels were normalized by deletion-mutated cRNA amplified at same time. \(^*\) \(P < .05\) vs sham-operated WT mice. \(\Delta\)AT\(^1\) and \(\Delta\)AT\(^2\) indicate amplified DNA from deleted cRNA as internal controls. Molecular weight marker (MWM) is also shown. (Fig 2B). Microscopic analysis revealed that cross-sectional areas of cardiac myocytes were increased only in WT mice (Fig 2C). In addition, Ang II treatment induced upregulation of the ANP gene only in WT mice (Fig 2D). These results strongly suggest that in KO hearts, Ang II does not evoke enough signals through AT\(^1\) to lead to the development of cardiac hypertrophy.
Effects of Pressure Overload on Expression of Fetal-Type Cardiac Genes

Upregulation of fetal-type genes and downregulation of the SERCA gene are well-established genetic responses to pressure overload produced by abdominal aortic banding. Ang II has been reported to be involved in the reprogramming of gene expressions induced by mechanical stress. We therefore examined the expression of these genes in hearts of WT and KO mice. Abdominal aortic banding induced upregulation of fetal-type cardiac genes such as ANP, BNP, and β-MHC genes and downregulation of SERCA gene in hearts of both mice (Fig 4). There were no significant differences in expression levels of these genes between WT and KO mice (Fig 4). These results suggest that pressure overload induces the reprogramming of gene expression in the heart irrespective of the presence of AT1-mediated Ang II signaling.

Morphological and Functional Changes in Hypertrophied Hearts

To determine the changes in left ventricular size and function, transthoracic echocardiography was performed before and 40 days after aortic banding. The left ventricular wall was

Figure 2. Effects of chronic Ang II administration. Subpressor dose of Ang II (100 ng · kg⁻¹ · min⁻¹ SC) was continuously infused into mice by an osmotic minipump for 14 days. A, Arterial blood pressure. *P<.005 vs WT mice. B, Heart weight (mg)/body weight (g) ratio. *P<.05 vs WT sham-operated mice. C, Cross-sectional areas of cardiac myocytes before and 14 days after Ang II infusion. Normalized values in WT mice before pressure overload are arbitrarily expressed as 1.0. *P<.001 vs sham-operated mice of each group. D, Representative autoradiograms of Northern blot analysis of ANP gene. Similar results were obtained from three independent experiments. Ethidium bromide staining of 18S ribosomal RNA is presented to show that loaded mRNA is equal and intact. Sham indicates sham-operated mice; Ang II, Ang II–infused mice.
thicker and left ventricular internal dimension was significantly larger in pressure overloaded hearts than in sham-operated hearts of both WT mice and KO mice (Fig 5, Table). In addition, almost the same degree of decrease in percent fractional shortening and ejection fraction was observed in both animal groups (Table).

Histological Changes in Hypertrophied Hearts

It has been reported that Ang II plays an important role in the development of ventricular remodeling, including cardiomyocyte hypertrophy and fibrosis, through AT1. Microscopic analysis revealed that cross-sectional areas of cardiac myocytes were increased both in KO mice and in WT mice (Fig 6A), indicating that each cardiac myocyte was increased in size by pressure overload without AT1 signaling. Perivascular fibrosis was prominent after pressure overload in KO hearts as well as in WT hearts (Fig 6B). The fibrotic area in KO hearts was almost the same as that in WT hearts after pressure overload (Fig 6C). In addition, the thickness of arterial walls was also increased in hearts of both kinds of mice (Fig 6B). These results suggest that pressure overload induces not only cardiac hypertrophy but also myocardial fibrosis, even when the heart lacks signaling through AT1.

Discussion

Many studies have demonstrated that treatment with ACE inhibitors or AT1 antagonists efficiently prevents and/or reduces pressure overload–induced cardiac hypertrophy in both experimental and clinical studies. In addition, all components of the RAS are upregulated by pressure overload in the heart. All these results suggest that the RAS plays a critical role in the development of mechanical stress–induced cardiac hypertrophy. In the present study, however, we have demonstrated that hemodynamic overload fully induces cardiac hypertrophy in genetically engineered mice that lack the AT1A gene, suggesting that AT1-mediated Ang II signaling is not indispensable for the development of pressure overload–induced cardiac hypertrophy.

We have developed an in vitro system of stretching cardiac myocytes cultured on deformable silicon dishes and have...
demonstrated that mechanical stretch induces activation of the protein kinase cascade of phosphorylation, transient expression of immediate early response genes, and an increase in protein synthesis, resulting in cardiomyocyte hypertrophy.17–21 These hypertrophic responses to mechanical stretch were inhibited by pretreatment with AT1 antagonists,22,23 suggesting that the endogenous Ang II secreted in response to mechanical stretch mediates the signaling pathway, leading to the development of cardiomyocyte hypertrophy in vitro. However, because treatment with AT1 antagonists only partially inhibited the hypertrophic responses to mechanical stretch in cardiac myocytes, signaling pathways other than those provoked by Ang II seem to be involved in the formation of cardiac hypertrophy.22 In addition, it has been reported that there are both common and divergent pathways between Ang II–and mechanical stretch–induced signaling pathways.22,25,26 These results, together with the findings that several humoral factors other than Ang II can induce cardiac hypertrophy,7,24,49 suggest that AT1A-mediated Ang II signaling is not the only essential factor for the development of mechanical stress–induced cardiac hypertrophy.

Recent progress in mouse genetics has brought about great advances in the understanding of regulatory mechanisms of the systemic circulatory system. Targeted gene disruptions of angiotensinogen,50 ACE,51 AT1A,29,30 and AT231,32 have indicated that the RAS plays a critical role in maintaining the homeostasis of systemic circulation. In mice lacking the AT1A gene, blood pressure was low and was not elevated with the administration of Ang II (Fig 2A), suggesting that AT1A plays a critical role in the Ang II–mediated regulation of blood pressure. Although the RAS is highly activated in AT1A KO mice, any histological abnormalities are not detectable in the heart.29,30 This study also demonstrates that slightly expressed AT1B was not functional in the development of Ang II–induced cardiac hypertrophy (Fig 2B through 2D). Although our experiments do not prove the role of AT1B, these findings suggest that AT1 is not involved in the development of cardiac hypertrophy in AT1A KO mice. Therefore, we tried to dissect the roles of AT1-dependent and -independent signaling pathways in the development of mechanical stress–induced cardiac hypertrophy by creating pressure overload in AT1A KO mice. At 40 days after abdominal aortic banding, pressure overload induced almost the same degree of cardiac hypertrophy in both KO and WT mice, which is consistent with a recent report.52 Cardiac hypertrophy in KO mice was

<table>
<thead>
<tr>
<th>Parameters in WT and KO Mice</th>
<th>WT</th>
<th>AT1A KO</th>
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<tbody>
<tr>
<td><strong>Sham</strong></td>
<td><strong>Banding</strong></td>
<td><strong>Sham</strong></td>
</tr>
<tr>
<td>IVST, mm</td>
<td>0.55±0.02</td>
<td>0.74±0.05*</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>0.55±0.02</td>
<td>0.81±0.06*</td>
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<tr>
<td>EDD, mm</td>
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<tr>
<td>ESD, mm</td>
<td>1.14±0.2</td>
<td>1.57±0.1*</td>
</tr>
<tr>
<td>%FS</td>
<td>63.7±3.0</td>
<td>53.7±3.0*</td>
</tr>
<tr>
<td>EDV, μm³</td>
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<td>99.0±8.0</td>
</tr>
<tr>
<td>ESV, μm³</td>
<td>5.4±1.0</td>
<td>13.0±3.0*</td>
</tr>
<tr>
<td>EF</td>
<td>0.944±0.02</td>
<td>0.879±0.03*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>321±48</td>
<td>300±25</td>
</tr>
</tbody>
</table>

IVST indicates intraventricular septum thickness; PWT, posterior wall thickness; EDD, end-diastolic diameter; ESD, end-systolic diameter; FS, fractional shortening; EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction; and HR, heart rate.

Figure 6. Light microscopic analysis. A, Cross-sectional areas of cardiomyocytes before and 40 days after aortic banding. Normalized values in WT mice before pressure overload are arbitrarily expressed as 1.0. *P<.001 vs sham-operated mice of each group. B, Histological examination of left ventricular fibrosis (van Gieson staining). Perivascular collagen accumulation (red) is prominent in both hearts after pressure overload. Original magnification, ×160. C, Relative area of fibrosis before and 40 days after aortic banding. Normalized values in sham-operated heart are arbitrarily expressed as 1. *P<.001 vs sham-operated mice of each group.
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associated with the reexpression of fetal-type cardiac genes and ventricular remodeling, such as perivascular fibrosis. Unexpectedly, in the abdominal aortic banding model, all these changes were indistinguishable between WT hearts and KO hearts. Although these results do not rule out the possibility that Ang II plays a critical role in forming cardiac hypertrophy in a physiological context, it strongly suggests that cardiac hypertrophy can be induced by pressure overload without Ang II signaling through AT₁A. Therefore, we speculate that blockade of AT₁ with pharmacological agents may not have any effects on the development of cardiac hypertrophy in KO mice.

This study suggests that there are two distinct pathways, AT₁-dependent and AT₁-independent pathways, in pressure overload–induced cardiac hypertrophy and that the AT₁-independent pathway can fully substitute for the AT₁-dependent pathway. We recently observed that acute pressure overload produced by transverse aortic banding could induce acute hypertrophic responses in KO mice as well as WT mice. It remains to be determined what factors substitute for AT₁-mediated signaling in the development of cardiac hypertrophy in AT₁A KO mice. We have demonstrated that ET-1 is involved in stretch-induced cardiomyocyte hypertrophy in vitro. Cultured cardiac myocytes secrete ET-1 in response to passive mechanical stretch, and ET₁ receptor activation mediates the stretch-induced hypertrophic responses. In our present experimental model, endogenous ET-1 may substitute for Ang II in the development of cardiac hypertrophy. Moreover, we have observed that passive stretch induced hypertrophic responses in cultured cardiac myocytes prepared from KO mice as well as WT mice and that tyrosine kinase inhibitors potently inhibited the stretch-induced responses in KO cardiac myocytes but not in WT cardiomyocytes. These results suggest that some humoral factors that activate tyrosine kinases are secreted in response to mechanical stress or that tyrosine kinase pathways, which are usually inhibited by AT₁-mediated signals, may be activated in the absence of AT₁A.

In summary, by using AT₁A KO mice, we demonstrated that AT₁A-mediated Ang II signaling is not indispensable for the development of pressure overload–induced cardiac hypertrophy and that signaling pathways other than those provoked by Ang II can fully induce hypertrophic responses during hemodynamic overload in the absence of AT₁. The identification of the AT₁-independent signaling pathways that are involved in pressure overload–induced cardiac hypertrophy will provide new insights into the development of novel therapeutic strategies for cardiac hypertrophy.

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References


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