Expression and localization of angiotensin subtype receptor proteins in the hypertensive rat heart

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Ozono, Ryoji, Toshiyuki Matsumoto, Tetsuji Shingu, Tetsuya Oshima, Yasuhiro Teranishi, Masayuki Kambe, Hideo Matsuura, Goro Kajiyma, Zhi-Qin Wang, Allan F. Moore, and Robert M. Carey. Expression and localization of angiotensin subtype receptor proteins in the hypertensive rat heart. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R781–R789, 2000.—The cellular localization of the AT2 receptor and the regulation of its expression in hypertrophied left ventricle are not well known. We compared the expression of the cardiac AT1 and AT2 receptor in spontaneously hypertensive rats/Izumo strain (SHR/Izm) and Wistar Kyoto rats/Izumo strain (WKY/Izm), ages 4, 12, and 20 wk, by means of immunohistochemistry and Western blot analysis. In SHR/Izm, compared with WKY/Izm, blood pressure (161 ± 2 vs. 120 ± 2 mmHg at 12 wk, P = 0.01, and 199 ± 3 vs. 123 ± 3 mmHg at 20 wk, P = 0.01) and heart-to-body weight ratio (3.76 ± 0.07 vs. 3.06 ± 0.06 mg/g at 12 wk, P = 0.01, and 3.90 ± 0.08 vs. 3.01 ± 0.12 mg/g at 20 wk, P = 0.01) were significantly elevated. There was no difference in these values between the two strains at 4 wk of age. Histologically, 20-wk-old SHR/Izm demonstrated myocardial hypertrophy, a thickening of the smooth muscle layer of the intracardiac arteries, and perivascular fibrosis. By immunohistochemistry, the AT2 receptor was localized to cardiomyocytes and vascular endothelial cells, but not in the vascular smooth muscle cells. No major AT2 receptor signal was observed in perivascular fibrosis at any age in either strain of rats. No difference was detected in this localization between the two strains. By Western blotting, a single 44-kDa band for the AT2 receptor and a single 60-kDa band for the AT1 receptor were detected in ventricles from both strains of rats at all ages. Densitometric analysis demonstrated that the AT2 receptor 44-kDa band was decreased by 20% at 12 wk and 32% at 20 wk (P < 0.01) in SHR/Izm compared with WKY/Izm. The intensity of the AT1 receptor 60-kDa band was increased by 57% in 20-wk-old SHR/Izm compared with WKY/Izm (P < 0.05). There was no significant difference in the intensity of the 44- or 60-kDa bands in 4-wk-old animals of either strain. We demonstrated a decrease in the AT2 receptor and an increase in the AT1 receptor protein with no change in their localizations in hypertrophied left ventricular myocytes of SHR/Izm.

Cardiac hypertrophy is an important risk factor for sudden cardiac death and ischemic heart disease. The heart expresses angiotensinogen (50), renin (3, 6), angiotensin-converting enzyme (37, 50), and both the AT1 (7, 15, 24, 36, 40) and AT2 receptors (17, 36, 40, 44, 48) in the heart of rats (17, 18, 24, 36, 40, 48), hamsters (26), rabbits (33), and humans (1, 25, 47), but its cellular localization and physiological or pathological significance still remain unclear. Also, it has not been confirmed whether this AT2 receptor is upregulated (17, 40) or downregulated (25) in cardiac hypertrophy.

The heart consists of myocytes and nonmyocytes, mostly fibroblasts. Pressure overload-induced left ventricular hypertrophy (45) and acute myocardial infarction (19) are associated with remodeling of cardiac structure, with development of perivascular and interstitial fibrosis. Investigators have focused on the effects of angiotensin II (ANG II) exerts its biological effects by binding to angiotensin receptors. Two major subtypes of angiotensin receptors, AT1 and AT2, have been recognized by ligand binding studies. AT1 receptors mediate most of the well-known ANG II effects in the cardiovascular system. The function of the AT2 receptor, on the other hand, has not been determined, although genomic (14) as well as cDNA (10, 20) sequences encoding the rat AT2 receptor have recently been elucidated. Recent studies have suggested that stimulation of the AT2 receptor has anti-proliferative effects on the neointima after vascular injury (22) and in coronary endothelial cells (39). The AT2 receptor also has been demonstrated to mediate inhibition of ANG II-induced-hypertrophy in cultured myocytes (4). Furthermore, the AT2 receptor is involved in the induction of apoptosis (49) and activation of tyrosine phosphatase (41). These observations support the hypothesis that the AT2 receptor is coupled to an antigrowth process that counteracts the growth-promoting program initiated by AT1 receptor activation (4).
of ANG II as a mediator of this remodeling process (19, 34, 45). It has been suggested (34, 35) that AT1 receptors play a major role in the transduction of the proliferative signal (18). However, a recent study (26) indicates that AT2 receptors are reexpressed in cardiac fibroblasts in failing heart and contribute to the inhibition of the proliferative process. It has not been studied whether AT2 receptor is expressed in fibroblasts in hypertrophied heart.

Recently, we generated a polyclonal antiserum against the rat AT2 receptor (30, 44) and demonstrated immunohistochemical localization of the receptor subtype in adult rat heart (44). In the present study, we investigated the distribution of the AT2 receptor by immunohistochemistry, for the first time to our knowledge, in the hearts of spontaneously hypertensive rats (SHR) with left ventricular hypertrophy. The cellular localization of this receptor, although providing no direct information on its function, is a necessary first step to begin to clarify the role of the AT2 receptor in the development of cardiac hypertrophy and remodeling. As a model of left ventricular hypertrophy, we used hearts from SHR, which provide an established model of left ventricular hypertrophy associated with systemic hypertension.

**METHODS**

**Animals.** SHR and normotensive controls, Wistar Kyoto rats/laboratory strain (WKY/Lzm), at ages of 4 (n = 10, each strain), 12 (n = 8, each strain), and 20 (n = 8, each strain) wk, were purchased from Disease Model Cooperative Research Association (Kyoto, Japan). Nara et al. (23) developed these inbred SHR and WKY strains that have almost identical genetic backgrounds. Systolic blood pressure was measured by the tail cuff method, as described previously (27). Animals were deeply anesthetized with pentobarbital sodium, and the hearts were freshly removed and weighed. The heart weight (mg) divided by the body weight (g) was considered a measure of ventricular hypertrophy.

**AT2-receptor antiserum.** Polyclonal antiserum was raised against a synthetic peptide sequence derived from the amino terminus of the predicted rat AT2 receptor (MKDNFSFAATSRRNITSS) (30, 44). The IgG fraction of the serum was obtained using protein A column as described previously (29). The protein concentration of the purified serum was 3.1 mg/ml, as determined by the Bradford method. Selectivity of the antiserum to the rat AT2 receptor was fully evaluated and published elsewhere (30, 44). Briefly, the antiserum recognized the AT2 receptor expressed in a stably transfected COS-7 cell line in Western blotting as a single 44-kDa band, whereas no band was observed in the nontransfected cell line. In the immunohistochemistry, the antiserum positively stained the transfected COS-7 cells grown on slides, whereas no signal was observed in nontransfected cells or in the transfected cells when reacted with the antiserum preadsorbed with its pure peptide immunogen (29, 44).

Rabbit anti-AT1 receptor polyclonal antiserum (AB1525), which is directed toward the carboxy terminal of the native receptor, was purchased from Chemicon International. The specificity was evaluated elsewhere (31).

**Histological and immunohistochemical analysis.** For histological demonstration of cardiac hypertrophy and remodeling, freshly removed ventricles from 20-wk-old SHR/Lzm and WKY/Lzm were immersion fixed in phosphate-buffered-10% formaldehyde solution and embedded in paraffin. Two-micrometer-thick sections were cut and processed according to an Elastica van Gieson staining protocol, which stains collagen a reddish-purple color. For analysis of ventricular myocyte cross-sectional area, microscopic fields were randomly selected from both epicardial and endocardial portions of ventricles and the images were acquired with a video camera (3 CCD color video camera KYF55B, Victor). The myocyte cross sections were traced, and the area was calculated with National Institute of Health (NIH) Image software program.

**Immunohistochemistry.** Immunohistochemistry was performed in frozen sections as described previously (29, 30, 44). Briefly, the hearts from the 4-, 12-, and 20-wk-old animals were immediately immersion fixed in 2% paraformaldehyde in PBS for 1–2 h. The tissue was cryoprotected overnight at 4°C in 30% sucrose in PBS, and frozen sections (6–8 mm) were cut. The sections were stored at −80°C until use. For the AT1 receptor staining, the endogenous peroxidase was quenched with 1% H2O2 in methanol, then the nonspecific binding sites of site 1) avidin, 2) biotin, and 3) secondary goat antibody were blocked with 1) avidin solution for 15 min, 2) biotin solution for 15 min (avidin biotin blocking kit, Vector Lab), and 3) 10% normal goat serum and 1% nonfat dry milk in PBS for 45 min, respectively. For the AT2 receptor staining, the endogenous peroxidase was quenched with 0.3% H2O2 in methanol, then the nonspecific binding sites of secondary goat antiserum were blocked with 3% normal goat serum and 2% nonfat dry milk in PBS for 45 min. The sections for both AT1 and AT2 receptors were then incubated overnight at 4°C with one of the following: 1) AT1 or AT2 receptor antiserum, 2) the IgG fraction of the preimmune serum, or 3) the antiserum against the AT2 receptor preadsorbed with its pure peptide antigen. Sera were diluted at 1:500 in 1.5% normal goat serum and 1% nonfat dry milk in PBS for AT2 receptor and 1:1,000 in 10% normal goat serum and 1% nonfat dry milk for AT1 receptor. In the preadsorption of the AT2 receptor antiserum, the serum was incubated for 24–48 h at 4°C with the immunizing peptide at 10-fold molar excess. Staining was visualized with the avidin-biotin immunoperoxidase reaction (Vectastain ABC Kit) using diaminobenzidine (Fast DAB tablets, Sigma) according to the manufacturer’s instructions.

Western blot analysis of AT1 and AT2 receptor protein expression. AT2 receptor protein expression in the heart was compared between SHR/Lzm and WKY/Lzm by Western blot analysis. Samples were prepared as previously described (29, 30, 44). Tissues were homogenized with Polytron in buffer A (10% glycerol, 20 mM Tris-HCl, 100 mM NaCl, 2 mM phenylmethylsulfonyl fluoride, 2 mM EDTA, 2 mM EGTA, 10 μg/ml leupetin, 10 μg/ml aprotinin, and 10 μg/ml pepstatin A). The homogenate was centrifuged at 30,000 g for 30 min at 4°C. The pellet was resuspended in buffer B (buffer A with 1% Nonidet P-40), stirred for 1 h at 4°C, and centrifuged again at 30,000 g. The supernatant was used for the analysis. The solubilized samples were subjected to SDS-PAGE (10% running gel). For comparison of the AT1 and AT2 receptor protein expression level between SHR/Lzm and WKY/Lzm, precisely 50 μg protein was loaded per gel. The protein concentration was determined by the bicinchoninic acid method. The resolved proteins were transferred onto a nitrocellulose membrane [Hi-bond enhanced chemiluminescence (ECL), Amersham] by electroblotting at 15 V for 20 min (Transblot SD DNA, Bio-Rad). The nitrocellulose membrane was soaked in Tris-buffered saline (TBS: 10 mM Tris-HCl, 150 mM NaCl) containing 5% nonfat dry milk (Skim Milk, Snow Brand) and 0.1% polyoxyethylene-sorbitan monolaurate (Tween 20) overnight at 4°C to block nonspecific sites, and then incubated with the AT1 or AT2 receptor antiserum.
(1:1,000 dilution in TBS with 5% nonfat dry milk and 0.1% Tween 20) for 2 h, and reacted with a peroxidase-conjugated donkey anti-rabbit secondary antibody (1:5,000 dilution) for 1 h. Immunoreactivity was visualized with an ECL Western Blotting Detection Kit (Amersham). NIH Image software program analyzed the intensity of the band. The bands were area traced, the size and the mean density were analyzed, and the product of the density by the area was defined as a band intensity that reflects the amount of protein and was expressed as arbitrary units.

Statistical analysis. Results were expressed as means ± SE. Change within a group was analyzed by ANOVA for repeated measures. Comparisons between SHR/Izm and WKY/Izm in the same age were made with two-tailed unpaired Student’s t-test. A value of P < 0.05 was accepted as statistically significant.

RESULTS

Validation of hypertension and left ventricular hypertrophy in SHR/Izm. As shown in Table 1, systolic blood pressure and the ratio of heart weight to body weight were significantly increased in 12- and 20-wk-old SHR/Izm compared with age-matched WKY/Izm, whereas no difference was detected in 4-wk-old animals. Histologically (Fig. 1), the ventricles of 20-wk-old SHR/Izm demonstrated characteristic signs of left ventricular hypertrophy, including increased cardiomyocyte diameter, perivascular fibrosis, and thickening of the vascular smooth muscle cell layer of the small coronary arteries. The mean values of ventricular myocyte cross-sectional areas in 20-wk-old SHR/Izm and WKY/Izm were 349 ± 12 and 276 ± 8 µm², respectively (P < 0.01). Interstitial fibrosis, which characterizes decompensated cardiac dysfunction, was present but not severe (Fig. 1). Similar, but less marked, histological findings were also observed in 12-wk-old SHR/Izm (data not shown).

Immunohistochemistry of the AT₂ receptor protein. In both SHR/Izm and WKY/Izm at all ages, the AT₂ receptor immunohistochemical signal was detected throughout the myocardium of left ventricle (Figs. 2 and 3), right ventricle, and atria (data not shown). The myocardial staining was homogeneous. Intracardiac small vessels were positively stained, but it was indistinguishable whether the staining was from vascular endothelium or vascular smooth muscle (data not shown). On the other hand, the vascular smooth muscle layer in the relatively large coronary artery (Fig. 3, broken arrow) and ascending aorta (data not shown) was clearly negative. In such large coronary arteries, the endothelium was positively stained (Fig. 3, arrowhead). The fibrous tissues were not remarkably stained, but a positive signal was observed in some perivascular fibrotic areas (Figs. 2 and 3, arrow).

Table 1. Body weight, heart weight, and blood pressure in 4-, 12-, and 20-week-old SHR/Izm and WKY/Izm

<table>
<thead>
<tr>
<th></th>
<th>SHR/Izm</th>
<th>WKY/Izm</th>
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<tbody>
<tr>
<td>4 wk</td>
<td>Body wt, g</td>
<td>106 ± 7</td>
</tr>
<tr>
<td></td>
<td>Heart wt, mg</td>
<td>430 ± 10</td>
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<tr>
<td></td>
<td>Heart wt/body wt, mg/g</td>
<td>4.19 ± 0.25</td>
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<tr>
<td></td>
<td>Blood pressure, mmHg</td>
<td>111 ± 3</td>
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<tr>
<td></td>
<td>Heart rate, beats/min</td>
<td>415 ± 13</td>
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<tr>
<td>12 wk</td>
<td>Body wt, g</td>
<td>246 ± 6*†</td>
</tr>
<tr>
<td></td>
<td>Heart wt, mg</td>
<td>927 ± 38*†</td>
</tr>
<tr>
<td></td>
<td>Heart wt/body wt, mg/g</td>
<td>3.76 ± 0.07*†</td>
</tr>
<tr>
<td></td>
<td>Blood pressure, mmHg</td>
<td>161 ± 2*†</td>
</tr>
<tr>
<td></td>
<td>Heart rate, beats/min</td>
<td>353 ± 9</td>
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<tr>
<td>20 wk</td>
<td>Body wt, g</td>
<td>398 ± 19*</td>
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<tr>
<td></td>
<td>Heart wt, mg</td>
<td>1548 ± 60*</td>
</tr>
<tr>
<td></td>
<td>Heart wt/body wt, mg/g</td>
<td>3.90 ± 0.08*†</td>
</tr>
<tr>
<td></td>
<td>Blood pressure, mmHg</td>
<td>199 ± 3*</td>
</tr>
<tr>
<td></td>
<td>Heart rate, beats/min</td>
<td>384 ± 15</td>
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Values are means ± SE. SHR/Izm, spontaneously hypertensive rats/Izumo strain; WKY/Izm, Wistar Kyoto rats/Izumo strain. *P < 0.01 vs. 4-wk-old rats; †P < 0.01 vs. WKY/Izm at same age.
the staining in the cardiomyocyte, connective tissue, and vascular endothelium between SHR/Izm and WKY/Izm at any age.

Figure 4 shows AT\textsubscript{1} receptor staining in the left ventricles of 20-wk-old SHR/Izm and WKY/Izm. AT\textsubscript{1} receptor was observed in cardiomyocytes, the vascular smooth muscle cells (Fig. 4, broken arrows), and perivascular tissue (Fig. 4, arrows) in both strains at all ages. No significant difference in the intensity or distribution of the signal was detected between the two strains.

Western blot analysis. A single 44-kDa band was observed in Western blots of the AT\textsubscript{2} receptor transfected COS-7 cells, but not in the nontransfected COS-7 cells (Fig. 5A). The same molecular weight band was seen in the ventricles at every age and strain. The approximate molecular mass of the AT\textsubscript{2} receptor was consistent with the calculated mass based on the molecular sequence and with that previously reported by our group (30, 44) and others (32). Densitometric analysis of the 44-kDa band (Fig. 5B) demonstrated...
that the band intensity was smaller in SHR/Izm compared with WKY/Izm by 7% at 4 wk, 5% at 12 wk, and 4% (P = 0.01) at 20 wk (Table 2). When only the right ventricles from 20-wk-old animals were used, a similar decrease in the 44-kDa band in SHR/Izm compared with WKY/Izm was observed (n = 2). The amount of AT2 receptor signal for SHR/Izm was significantly smaller at 20 wk of age than at 4 wk of age (P < 0.05). In WKY/Izm, the intensities of the bands were not significantly changed by the age (Table 2).

The AT1 receptor was also detected by use of anti-AT1 receptor antiserum (Fig. 6A). The approximate molecular mass was 60 kDa, consistent with the reported value (13, 21, 31). The densitometric analysis revealed that the band intensity was greater in SHR/Izm by 6% at 12 wk and 12% (P = 0.05) at 20 wk than WKY/Izm at the same age (Fig. 6B), whereas no significant difference was detected in 4-wk-old animals (Table 2).

**DISCUSSION**

In the present study, we demonstrated that 1) SHR/Izm, at ages of 12 and 20 wk, had left ventricular hypertrophy accompanied by remodeling of the connective tissue; 2) the expression of AT2 receptor protein in ventricular myocytes was decreased, whereas that of the AT1 receptor was increased in 12- and 20-wk-old SHR/Izm compared with WKY/Izm at the same age; and 3) there was no remarkable AT2 receptor staining in perivascular and interstitial connective tissues, and the staining intensity was not changed during the development of the cardiac remodeling. There had been no immunohistochemical studies localizing both AT1 and AT2 receptors in diseased heart. Recent studies indicated that AT2 receptors are locally reexpressed in the sites of cellular hyperplasia in the failing heart (26, 47) or in wound healing (42). In left ventricular hypertrophy in SHR/Izm, however, such a local accumulation of the receptor subtype was not observed until at least 20 wk of age.

It is tempting to speculate that the balance of the expression of two receptor subtypes may determine the overall activity of ANG II in the heart, and the decrease in the AT2 receptor may contribute to development of left ventricular hypertrophy in SHR. However, we cannot conclude this from our present observations, because we only examined change in the receptor number but not change in receptor-agonist interaction or receptor-mediated signal transduction. Up- or down-regulation of the receptor does not always parallel the change in the activity of the receptor system. In addition, whether the change in the ratio of AT1/AT2 receptor can modulate development of left ventricular hypertrophy also needs to be established by functional studies.

The mechanism for the regulation of AT1 and AT2 receptor expression is not well understood. Mechanical stretch of myocardium (13, 34) causes enhanced ANG II production and upregulation of AT1 receptor. The latter finding was consistent with our observations, Wang et al. (43) recently reported that administration of ANG II downregulated AT1 receptors but had no effect on AT2 receptors in the kidney. It is unclear whether the changes in the AT1 and AT2 receptors observed in the present study were caused by pressure overload or by other humoral factors including ANG II, because the mechanism of the left ventricular hypertrophy in SHR is multifactorial. However, we (28) recently observed that the AT2 receptor was uniformly downregulated in left ventricular hypertrophy regardless of the cause of hypertrophy, including coarctation of aorta, deoxycorti-
costerone-acetate salt hypertension, and two kidney, one-clip hypertension, indicating that the pressure overload is likely to be the major mechanism whereby this receptor subtype is downregulated.

As to the subcellular mechanism, the gene expression of the AT$_2$ receptor is also regulated by multiple factors. Increase in intracellular calcium level by ionophore (13) and activation of protein kinase C (PKC) by phorbol ester (13) and cAMP analog (16) downregulated the AT$_2$ receptor mRNA or AT$_2$ receptor binding in PC12 cells. Norepinephrine and ANG II, which elevate Ca$^{2+}$ levels and activate PKC, downregulated the AT$_2$ receptor in cardiac myocytes (12). Growth factors, including epidermal growth factor, nerve growth factor, and platelet-derived growth factor, also downregulated AT$_2$ receptor mRNA expression in PC12 cells (12) and R3T3 cells (8). On the other hand, Ichiki et al. (8, 9) and Kambayashi et al. (11) reported that AT$_2$ receptor mRNA is upregulated by interleukin-1$\beta$, insulin, and insulin-like growth factor. According to these observa-

![Image](https://api.repub.org/10.220.33.6/June.27.2017/DECREASED.AT2.RECEPTOR.IN.LEFT.VENTRICULAR.HYPERTROPHY)
tions, it is more conceivable that AT₂ receptor is down-regulated in left ventricular hypertrophy as observed in the present study, because PKC, cAMP, and growth factors are all increased in left ventricular hypertrophy.

Previously, Suzuki et al. (40) reported that both AT₁ and AT₂ receptor binding capacity and mRNAs detected by RT-PCR were increased in the heart with left ventricular hypertrophy from renovascular hypertensive rats as well as in SHR at ages 20 and 24 wk. Similarly, the same group (13) reported that mechanical stretch for several days in cultured cardiomyocytes upregulated AT₁ and AT₂ receptors via signals involving stretch-activated tyrosine kinases. In addition, Lopez et al. (17), using a ligand binding technique, observed an increase in the proportion of AT₂ receptor number relative to that of AT₁ receptor after 4 wk of treatment with aortic banding. However, Wolf et al. (48) reported that neither AT₁ nor AT₂ receptor message was affected by aortic banding for several weeks. Nozawa et al. (25), again using ligand binding technique, reported a downregulation of AT₂ receptor in left ventricular hypertrophy in humans. Therefore, it has not been conclusively determined whether AT₂ receptor is upregulated or downregulated in left ventricular hypertrophy. The discrepancy may be explained by differences in species of animals, pathological stage of hypertrophy. The AT₂ receptors were observed only in cardiomyocytes regardless of the hemodynamic and structural changes associated with the left ventricular hypertrophy. Also, we found that left ventricular hypertrophy in SHR/Izm was associated with the downregulation of the AT₂ receptor and upregulation of AT₁ receptor in cardiomyocytes. These findings provide important information for the investigation of the functional role of angiotensin receptors in left ventricular hypertrophy.

In summary, we localized AT₁ and AT₂ receptors in the heart of SHR/Izm with left ventricular hypertrophy. The AT₂ receptors were observed only in cardiomyocytes regardless of the hemodynamic and structural changes associated with the left ventricular hypertrophy. Therefore, it has been conclusively determined whether AT₂ receptor is increased or decreased in left ventricular hypertrophy.

It is known that left ventricular hypertrophy, in its late stage, progresses into heart failure associated with interstitial fibrosis, a loss of myocardial contractility, and an increase in cardiac diameter (5). It is possible that our observation in 20-wk-old SHR/Izm is, in part, related to heart failure rather than hypertrophy. However, the histological observation in 20-wk-old SHR/Izm demonstrates no evidence of transition of the hypertrophy to heart failure, including severe interstitial fibrosis and decrease in myocardial wall thickness. In addition, recent studies (1, 46) have demonstrated that the AT₁ receptor was downregulated in end-stage heart failure. In the present study, the AT₁ receptor was upregulated by ~50%. These observations suggest that the 20-wk-old SHR was in the stage of ventricular hypertrophy that preceded heart failure.

There is a substantial difference in the genetic backgrounds of conventional SHR and WKY. To circumvent this disadvantage of SHR, in the present study, we used a newly developed inbred strain of SHR/Izm and WKY/Izm, in which it has been established that the matching of the genetic backgrounds has been markedly improved (23). Similarly, observed changes in AT₁ and AT₂ receptors could be associated with a genetic defect in the regulation of the renin-angiotensin system that is unique to SHR, but has nothing to do with the hypertension. To study this possibility, we compared the change in AT₂ receptor density in other pressure overload models in rats (28) and obtained the same finding as in this study. Furthermore, in the present study, there was no difference in either the AT₁ and AT₂ receptor between the strains in prehypertensive 4-wk-old animals. Therefore, it is unlikely that the change in AT₁ and AT₂ receptors during the development of cardiac hypertrophy is independent of systemic hypertension.

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