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Effects of an AT_1 receptor antagonist, an ACE inhibitor and a calcium channel antagonist on cardiac gene expressions in hypertensive rats

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1 This study was undertaken to determine whether the AT_1 receptor directly contributes to hypertension-induced cardiac hypertrophy and gene expressions.

2 Stroke-prone spontaneously hypertensive rats (SHRSP) were given orally an AT_1 receptor antagonist (losartan, 30 mg $kg^{-1}day^{-1}$), an angiotensin converting enzyme inhibitor (enalapril 10 mg $kg^{-1}day^{-1}$), a dihydropyridine calcium channel antagonist (amlodipine, 5 mg $kg^{-1}day^{-1}$), or vehicle (control), for 8 weeks (from 16 to 24 weeks of age). The effects of each drug were compared on ventricular weight and mRNA levels for myocardial phenotype- and fibrosis-related genes.

3 Left ventricular hypertrophy of SHRSP was accompanied by the increase in mRNA levels for two foetal phenotypes of contractile proteins (skeletal α-actin and β-myosin heavy chain (β-MHC)), atrial natriuretic polypeptide (ANP), transforming growth factor-β-1 (TGF-β1) and collagen, and a decrease in mRNA levels for an adult phenotype of contractile protein (α-MHC). Thus, the left ventricle of SHRSP was characterized by myocardial transition from an adult to a foetal phenotype and interstitial fibrosis at the molecular level.

4 Although losartan, enalapril and amlodipine lowered blood pressure of SHRSP to a comparable degree throughout the treatment, losartan caused regression of left ventricular hypertrophy of SHRSP to a greater extent than amlodipine ($P<0.01$).

5 Losartan significantly decreased mRNA levels for skeletal α-actin, ANP, TGF-β1 and collagen types I, III and IV and increased α-MHC mRNA in the left ventricle of SHRSP. Amlodipine did not alter left ventricular ANP, α-MHC and collagen types I and IV mRNA levels of SHRSP.

6 The effects of enalapril on left ventricular hypertrophy and gene expressions of SHRSP were similar to those of losartan, except for the lack of inhibition of collagen type I expression by enalapril.

7 Unlike the hypertrophied left ventricle, there was no significant difference between losartan and amlodipine in the effects on non-hypertrophied right ventricular gene expressions of SHRSP.

8 Our results show that hypertension causes not only left ventricular hypertrophy but also molecular transition of myocardium to a foetal phenotype and interstitial fibrosis-related molecular changes. These hypertension-induced left ventricular molecular changes may be at least in part mediated by the direct action of local angiotensin II via the AT_1 receptor.

Keywords: AT_1 receptor; hypertension; left ventricular hypertrophy; cardiac phenotype; cardiac fibrosis; myosin heavy chain; α-actin; transforming growth factor-β1; collagen; gene expression

Introduction

Hypertension causes cardiac left ventricular hypertrophy, which is the major risk factor for the development of congestive heart failure (Frohlich, 1983; Kannel *et al.*, 1987). Pathological left ventricular hypertrophy, by pressure overload, is accompanied not only by quantitative change (myocyte hypertrophy) but also by qualitative changes, including the shift to a foetal phenotype of the myocytes (Schwartz *et al.*, 1986; Izumo *et al.*, 1988; Nadal-Ginard & Mahdavi, 1989; Parker & Schneider, 1991) and interstitial fibrosis (Doering *et al.*, 1988; Weber & Brilla, 1991). The re-expression of the foetal phenotype of contractile proteins, such as β-myosin heavy chain (MHC) and skeletal α-actin, in the myocytes significantly alters the myocardial contractility (Nadal-Ginard & Mahdavi, 1989; Hewett *et al.*, 1994 Dorn *et al.*, 1994). Furthermore, left ventricular interstitial fibrosis enhances abnormal myocardial stiffness, thereby leading to the ventricular diastolic dysfunction (Weber & Brilla, 1991). Thus, these qualitative changes as well as the quantitative change of the left ventricle may be involved in the transition from compensatory hypertrophy to ventricular failure. However, the characteristics and mechanism of hypertension-induced left ventricular hypertrophy at the molecular level remain to be elucidated.

Recent investigations using cultured cells from the neonatal rat heart, show that angiotensin II (AII) *in vitro* directly causes the hypertrophy of cardiac myocytes and the hyperplasia of cardiac fibroblasts via AT_1 receptors, and that AII *in vitro* induces the foetal phenotype of genes in cardiac myocytes via the AT_1 receptor (Sadoshima & Izumo, 1993). Furthermore, Sadoshima *et al.* (1993), using an *in vitro* model of load (stretch)-induced cardiac hypertrophy, have obtained direct evidence that mechanical stretch causes the release of a significant amount of AII from cardiac myocytes and the released AII acts as an initial mediator of the load-induced cardiac hypertrophic response. These *in vitro* findings, taken together with the existence of local renin-angiotensin system in the heart (Lindpaintner & Ganten, 1991), led us to propose the hypothesis that hypertension-induced left ventricular hypertrophy *in vivo* may be in part mediated by the autocrine action of AII. However, it is unknown whether or not the above mentioned *in vitro* action of AII on cardiac hypertrophy can apply to the *in vivo* situation.

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The present study was undertaken to determine whether the AT_1 receptor contributes directly to the development of hypertension-induced pathological left ventricular hypertrophy. For this purpose, we measured ventricular mRNA levels for cardiac hypertrophy-associated genes of hypertensive rats, and compared the effects of an AT_1 receptor antagonist (losartan), an angiotensin converting enzyme (ACE) inhibitor (enalapril) and a dihydropyridine calcium channel antagonist (amlodipine) on these gene expressions.

Methods

Experimental protocol

All procedures were in accordance with institutional guidelines for animal research. Male stroke-prone spontaneously hypertensive rats (SHRSP) (Okamoto *et al.*, 1974) and Wistar-Kyoto rats (WKY) as the genetic control, which were supplied by Ciba-Geigy Japan Limited (Takarazuka, Japan), were used in this study. Animals were housed at a constant temperature (23°C) with a 12 h light-dark cycle, fed a standard laboratory chow (CE2, Clea Japan, Tokyo, Japan) and given tap water *ad libitum*. Sixteen-week-old male SHRSP were randomly separated into 4 groups, including (1) losartan-treated, (2) enalapril-treated, (3) amlodipine-treated and (4) vehicle-treated groups. Each drug was suspended with 0.5% carboxymethylcellulose solution. Losartan (30 mg kg^{-1} day^{-1}), enalapril (10 mg kg^{-1} day^{-1}), amlodipine (5 mg kg^{-1} day^{-1}), and vehicle (0.5% carboxylmethylcellulose), in a volume of 2 ml kg^{-1}, were given to SHRSP by gastric gavage once a day for a period of 8 weeks (from 16 to 24 weeks of age). After 8 weeks of drug treatment, the above mentioned 4 groups of 24-week-old SHRSP and the same age of WKY ($n=7$ per group) were decapitated, and the heart was immediately excised from each rat. The left and the right ventricles were carefully separated from the atria, weighed, and immediately frozen in liquid nitrogen. The entire procedure was completed within 3 min. The tissues were stored at −80°C until the extraction of total RNA.

In another experiments, losartan (30 mg kg^{-1} day^{-1}), enalapril (10 mg kg^{-1} day^{-1}), or amlodipine (5 mg kg^{-1} day^{-1}) was given to SHRSP in the same manner as the above mentioned experiments for 8 weeks (from the age of 16 to 24 weeks), to compare their hypotensive effects in detail. On day 1, and 4 and 8 weeks after the start of the treatment, we measured blood pressure of SHRSP at 2, 5, 8 and 24 h after the oral administration of each drug.

Oligonucleotide and cDNA probes

Although there is a high degree of homology between the coding regions of rat α- and β-MHC mRNAs and between those of rat skeletal and cardiac α-actin mRNAs, the 3′ untranslated regions are not closely conserved between these MHC mRNAs (Mahdavi *et al.*, 1984; Gustafson *et al.*, 1985) and between these α-actin mRNAs (Shani *et al.*, 1981; Mayer *et al.*, 1984). Therefore, in the present study, to detect each mRNA specifically by Northern blot analysis, we used synthetic oligonucleotide probes complementary to the unique 3′ untranslated regions of these MHC and α-actin mRNAs, as described by Kim *et al.* (1995b). The sequences of oligonucleotide probes used were as follows: α-MHC, 5′-TTGTGGGATAGCAACAGCGA-3′; β-MCH, 5′-GTCTCAGGGCTTCACAGG-3′; skeletal α-actin, 5′-GCAACCATAGCACGATGGTC-3′; cardiac α-actin, 5′-TGCACGTGTGTAAACAAACT-3′. For hybridization, the oligonucleotide probes were labelled with (γ-^{32}P)-ATP (6,000 Ci $mmol^{-1}$) at the 5′ end, using T4 polynucleotide kinase, and then purified by chromatography on Bio-Spin 6 column (Bio-Rad, Richmond, U.S.A.).

cDNA probes used were as follows: rat TGF-β1 cDNA, a HindIII/XbaI fragment (Qian *et al.*, 1990); rat α1(I) collagen cDNA, a 1.3 kb PstI/BamHI fragment (Genovese *et al.*, 1984); mouse α1(III) collagen cDNA, a 1.8 kb EcoRI/EcoRI fragment (Liau *et al.*, 1985); mouse α1(IV) collagen cDNA, a 0.83 kb AvaI/PstI fragment (Oberbaumer *et al.*, 1985); rat atrial natriuretic polypeptide (ANP) cDNA, a 0.825 kb fragment, which was synthesized by the reverse-transcriptase polymerase chain reaction method (Nakayama *et al.*, 1984); rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA, a 1.3 kb PstI/PstI fragment (Fort *et al.*, 1985). For hybridization, the cDNA probes were labelled with [^{32} P]-dCTP (specific activity 3000 Ci $mmol.^{-1}$, New England Nuclear, Boston, U.S.A.) by random primer extension method (Feinberg & Vogelstein, 1983), using a Random Primer DNA Labelling Kit (Takara, Kyoto, Japan).

RNA extraction

Total RNA was isolated from the individual left and right ventricles, by the guanidinium thiocyanate-phenol-chloroform method (Chomczynski & Sacchi, 1987), as described by Kim *et al.* (1994). The RNA concentration was determined spectrophotometrically by absorbance at 260 nm.

Northern blot hybridization

Twenty μg of total RNA from the left or right ventricle was denatured in 1 M glyoxal and 50% dimethyl sulphoxide at 50°C for 1 h, separated on 1% agarose gel and transferred to a nylon membrane (Gene Screen Plus membrane, DuPont Co., Boston, MA, U.S.A.), as described by Kim *et al.* (1994). The 28S and 18S ribosomal RNAs in gels were stained with ethidium bromide, to demonstrate the integrity of applied RNA and to verify that the same amounts of RNA were applied to each lane. For hybridization with oligonucleotide probes, the membranes were prehybridized in a solution containing 20 mM NaH_2PO_4 (pH 7.4), 6 × SSC (1 × SSC = 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7), 5 × Denhardt's solution (Ficoll, polyvinylpyrrolidone and bovine serum albumin, 1 mg ml^{-1} each), 0.1% sodium dodecyl sulphate (SDS) and 200 μg ml^{-1} denatured salmon sperm DNA at 42°C for 4 h and then hybridized in the same solution as prehybridization solution, containing the radiolabelled oligonucleotide probes, at 42°C for 24 h. After hybridization, the membranes were washed in 2 × SSC for 10 min at room temperature. Then the membranes were further washed in 2 × SSC containing 1% SDS for 60 min, at different washing temperatures depending upon the oligonucleotide probes used: 55°C for α-MHC; 53°C for β-MHC; 57°C for skeletal α-actin; 51°C for cardiac α-actin. Finally, for all hybridizations to oligonucleotide probes, the membranes were washed in 0.1 × SSC at room temperature for 20 min. In the case of cDNA probes, all procedures, including prehybridization, hybridization and washing of the membranes, were carried out in the same conditions as described by Kim *et al.* (1994).

After washing, the membranes were exposed to X-ray films (Kodak X-Omat AR5, Eastman Kodak Co., Rochester, N.Y.) between two intensifying screens at −70°C. The density of mRNA bands, obtained by autoradiography, was measured by Macintosh LC-III computer with an optical scanner (EPSON GT-8000, Seoko, Tokyo, Japan), using the public domain NIH Image programme (Kim *et al.*, 1994). In all ventricular samples, the hybridization signals of specific mRNAs were normalized for those of GAPDH mRNA, to correct for differences in RNA loading and/or transfer. After autoradiography, the membranes were boiled in 0.1 × SSC containing 1% SDS for 30 min to strip off the hybridized oligonucleotide or cDNA probe, and were then rehybridized with other oligonucleotide or cDNA probes.

Measurement of blood pressure

Systolic blood pressure of conscious rats was measured by the tail cuff method using a sphygmomanometer (Riken development Co., Ltd., Tokyo, Japan). Blood pressure was measured

at 5 h after oral dosing of each drug or vehicle, because preliminary studies showed that the maximal hypotensive effect of the three drugs used occurs at about 5 h after oral dosing. Each value is the average of three consistent readings.

Drugs

The AT_1 receptor antagonist, losartan, was a gift from DuPont Pharmaceutical Co. (Wilimington, U.S.A.). The ACE inhibitor, enalapril maleate, was kindly provided by Banyu Pharmaceutical Co. (Tokyo, Japan). Losartan, enalapril and amlodipine (Fleckenstein *et al.*, 1989) were suspended with 0.5% carboxymethylcellulose.

Statistical analysis

Results were expressed as means ± s.e. mean. The data on blood pressure and heart rate, before and at 2, 4 and 8 weeks after start of drug treatment, were analyzed by two-way analysis of variance (ANOVA), and the differences between each group at each time point were determined by the least-squares means test (SuperANOVA, Abacus Concepts, Berkeley, U.S.A.). Statistical significance of the data on body weight, left and right ventricular weights and mRNA levels of 24-week-old WKY and SHRSP was determined by one way ANOVA and Duncan's multiple range test. Differences were considered statistically significant at a value of $P<0.05$.

Results

Effects of losartan, enalapril and amlodipine on blood pressure, heart rate and cardiac hypertrophy

As shown in Figure 1, our detailed experiments on blood pressure of SHRSP treated with losartan, enalapril and amlodipine showed that blood pressure was similar among these 3 groups of SHRSP on day 1, and at 4 and 8 weeks after the start of the treatment, thereby showing comparable hypotensive effects of these 3 drugs in SHRSP.

In the present study, as shown in Figure 2a, blood pressure of 16-week-old SHRSP was already significantly higher than in WKY rats of the same age ($P<0.01$). Before start of drug treatment, there was no difference in blood pressure among 4 groups of 16-week-old SHRSP, including vehicle, losartan, enalapril and amlodipine-treated groups (210 ± 5, 209 ± 4, 209 ± 4 and 209 ± 4 mmHg, respectively). Losartan, enalapril or amlodipine significantly lowered blood pressure of SHRSP throughout the treatment (from the age of 16 to 24 weeks) ($P<0.01$). There was no significant difference in hypotensive effects between losartan and enalapril throughout the treatment. However, amlodipine lowered blood pressure of SHRSP to a greater extent than losartan (163 ± 2 vs. 172 ± 6 mmHg, respectively; $P<0.05$) at 18 weeks of age, and to a greater extent than losartan (158 ± 3 vs. 179 ± 7 mmHg, $P<0.01$) and enalapril (173 ± 5 mmHg, $P<0.05$) at 24 weeks of age. Thus, our present observations on blood pressure of SHRSP were similar to the data from our other experiments shown in Figure 1. As shown in Figure 2b, there was no significant difference in heart rate among 16 week-old WKY and 4 groups of SHRSP before the start of drug treatment. At the age of 18, 20 or 24 weeks, heart rate of all 4 groups of SHRSP was significantly higher than that of WKY rats ($P<0.01$). At 24 weeks of age, there was only a slight increase in heart rate of SHRSP treated with losartan (417 ± 8 b.p.m.) and amlodipine (419 ± 18 b.p.m.) compared to vehicle-treated SHRSP (380 ± 23 b.p.m.) ($P<0.05$).

As shown in Table 1, left ventricular weight of 24-week-old vehicle-treated SHRSP was significantly higher than that of the same age of WKY ($P<0.01$). Treatment with losartan, enalapril or amlodipine caused significant regression of left ventricular hypertrophy in SHRSP ($P<0.01$). Furthermore, left ventricular weight, corrected for body weight, of losartan-treated SHRSP was significantly smaller than that of amlodi-

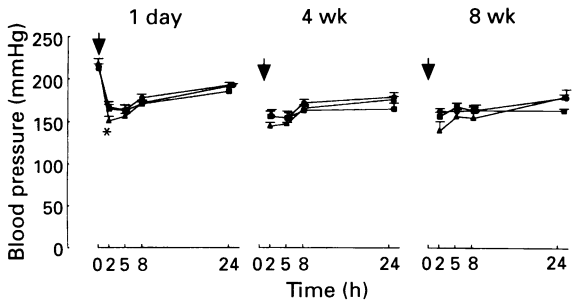

Figure 1 Hypotensive effects of losartan (□), enalapril (●) and amlodipine (▲) on SHRSP on day 1, and at 4 and 8 weeks after the start of the treatment. Data are expressed as means ± s.e.mean of 4 animals per group. Analysis of the data was carried out by two-way ANOVA, and the differences between each group at the same age were determined by the least-squares means test. *$P<0.05$ compared to losartan-treated and enalapril-treated groups of SHRSP. The arrows indicate the oral administration of each drug. One day, 4 wk and 8 wk show blood pressure on day 1 and at 4 and 8 weeks, respectively, after the start of drug treatment.

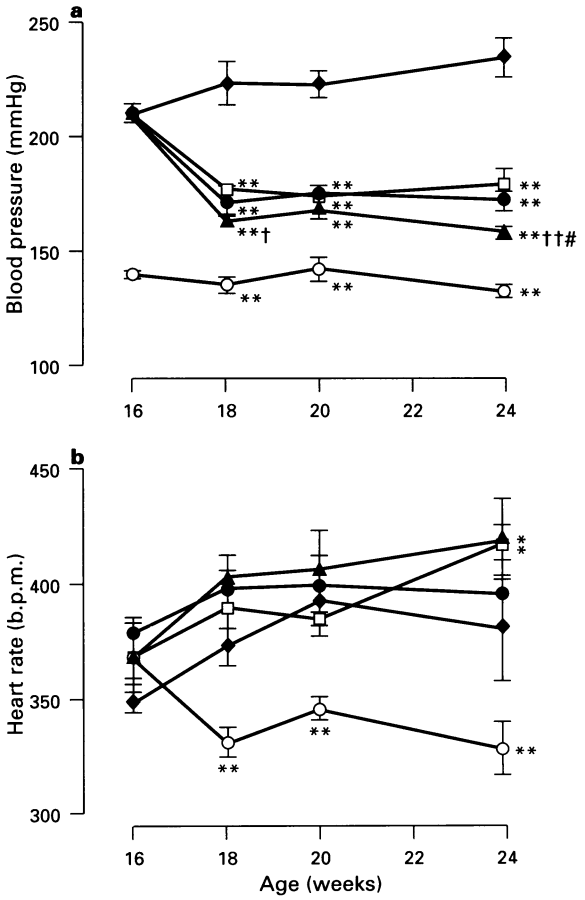

Figure 2 Blood pressure (a) and heart rate (b) of WKY (○), and SHRSP treated with vehicle (◆), losartan (□), enalapril (●) and amlodipine (▲). Data are expressed as means ± s.e. mean of 7 animals per group. Analysis of the data was carried out by two-way ANOVA, and the differences between each group at the same age were determined by the least-squares means test. (*$P<0.05$, **$P<0.01$ compared to vehicle-treated SHRSP. †$P<0.05$, ††$P<0.01$ compared to losartan-treated SHRSP. #$P<0.05$ compared to enalapril-treated SHRSP.

Table 1 Body weight, and left and right ventricular weights of 24-week-old WKY and SHRSP

Group (*n*)	*BW* (g)	*LV* (mg)	*RV* (mg)	*LV/BW* (mg g^{-1})	*RV/BW* (mg g^{-1})
WKY (7)	415±10**	780±22**	179±3	1.93±0.02**	0.433±0.018
SHRSP					
Vehicle (7)	350±5	1090±14	175±9	3.12±0.03	0.501±0.024
Losartan (7)	331±5	864±11**	180±8	2.61±0.02**	0.542±0.020
Enalapril (7)	338±5	921±27**†	166±10	2.74±0.11**	0.493±0.031
Amlodipine (7)	342±3	979±8**††#	188±5	2.86±0.03**††	0.550±0.020

BW, body weight; LV, left ventricular weight; RV, right ventricular weight. LV/BW and RV/BW indicate left and right ventricular weights, respectively, corrected for body weight. Vehicle, losartan, enalapril and amlodipine indicate SHRSP treatment with each for 8 weeks. Values are the mean±s.e.mean of 7 animals per group. Statistical significance was determined by one-way ANOVA. **$P<0.01$ compared to vehicle. †$P<0.05$, ††$P<0.01$ compared to losartan. #$P<0.05$ compared to enalapril.

pine-treated SHRSP ($P<0.01$). On the other hand, there was no significant difference in right ventricular weight, corrected for body weight or not, between WKY and vehicle-treated SHRSP. Losartan, enalapril or amlodipine did not significantly alter right ventricular weight of SHRSP.

Effects of losartan, enalapril and amlodipine on left ventricular gene expression

As shown in Figures 3 and 4, left ventricular skeletal α-actin, ANP, β-MHC and cardiac α-actin mRNA levels in vehicle-treated SHRSP were 5.1, 7.5, 1.6 and 1.2 fold, respectively, higher than those in WKY ($P<0.01$), while α-MHC mRNA levels of vehicle-treated SHRSP were decreased to 71% of those of WKY ($P<0.01$). Both losartan and enalapril decreased left ventricular skeletal α-actin ($P<0.01$) and ANP ($P<0.01$) mRNAs of SHRSP to a similar extent. On the other hand, amlodipine decreased skeletal α-actin mRNA to a smaller extent than losartan ($P<0.01$) and enalapril ($P<0.01$), and there was no decrease in ANP mRNA by amlodipine. β-MHC mRNA levels of SHRSP were not altered by these three drugs. α-MHC mRNA of SHRSP was elevated to similar levels to those of WKY by losartan ($P<0.01$) and to a partial extent by enalapril ($P<0.05$), but not elevated by amlodipine. Cardiac α-actin mRNA of SHRSP was significantly decreased only by treatment with losartan. As shown in Table 2 the ratio of left ventricular β-MHC to α-MHC mRNAs of vehicle-treated SHRSP was 2.3 fold higher than that of WKY ($P<0.01$). Losartan significantly decreased this ratio of SHRSP ($P<0.05$). Neither enalapril nor amlodipine decreased this ratio of SHRSP, although enalapril tended to decrease it.

As shown in Figures 5 and 6, mRNA levels for left ventricular TGF-β1 and collagen types I, III and IV of vehicle-treated SHRSP were 1.5, 2.0, 2.1 and 1.6 fold, respectively, larger than those of WKY ($P<0.01$). Losartan significantly decreased all these mRNA levels of SHRSP. Enalapril decreased TGF-β1, and collagen types III and IV, but did not decrease collagen type I mRNA. Amlodipine failed to lower collagen types I and IV mRNA levels of SHRSP.

Effects of losartan, enalapril and amlodipine on right ventricular gene expression

As shown in Figure 7, right ventricular skeletal α-actin, ANP and β-MHC mRNA levels of SHRSP were 4.1, 4.4 and 2.1 fold, respectively, higher than those in WKY ($P<0.01$), while α-MHC mRNA levels of SHRSP were decreased to 84% of those of WKY ($P<0.05$). There was no difference in cardiac α-actin mRNA levels between SHRSP and WKY. All three drugs significantly decreased skeletal α-actin mRNA of SHRSP ($P<0.01$), but the decreases induced by losartan or enalapril were greater than amlodipine ($P<0.05$). ANP mRNA of SHRSP was significantly decreased only by treatment with enalapril. β-MHC and α-MHC mRNAs of SHRSP were significantly decreased and increased, respectively, by losartan, enalapril or amlodipine. As shown in Table 2, the ratio of right ventricular β- to α-MHC mRNAs in SHRSP was greater than in WKY ($P<0.01$). All three drugs used significantly lowered this ratio in the right ventricle of SHRSP.

As shown in Figure 8, right ventricular TGF-β1 and collagen type IV mRNA levels of SHRSP were 1.3 and 1.4 fold respectively, higher than those of WKY. These mRNAs of SHRSP were significantly reduced by treatment with losartan, enalapril and amlodipine. Collagen types I and III mRNA values of SHRSP were not significantly different from WKY, and were not changed by the three drugs.

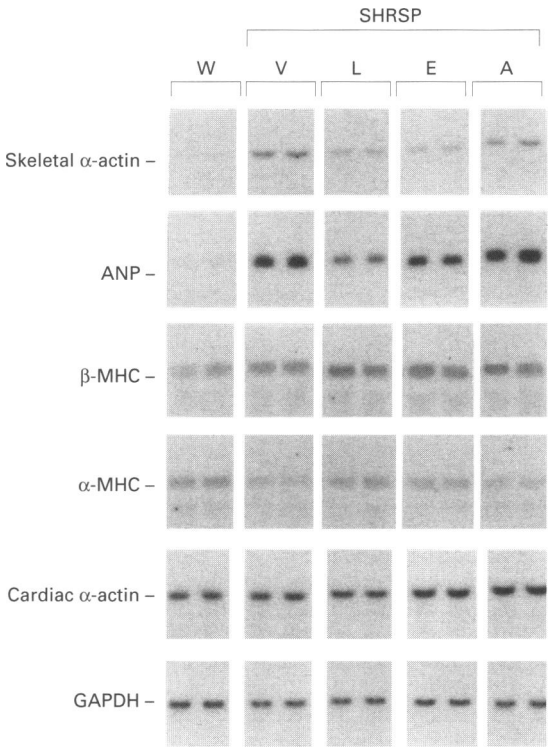

Figure 3 Typical autoradiograms of Northern blot analysis of left ventricular mRNAs for cardiac phenotype-related genes. W, WKY; V, vehicle-treated SHRSP; L, losartan-treated SHRSP; E, enalapril-treated SHRSP; A, amlodipine-treated SHRSP. The size of mRNA band was 1.7 kb for skeletal α-actin, 0.9 kb for atrial natriuretic polypeptide (ANP), 7.1 kb for β-myosin heavy chain (β-MHC), 7.1 kb for α-myosin heavy chain (α-MHC), 1.7 kb for cardiac α-actin, and 1.4 kb for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Autoradiograms of mRNAs from two different animals per group are shown.

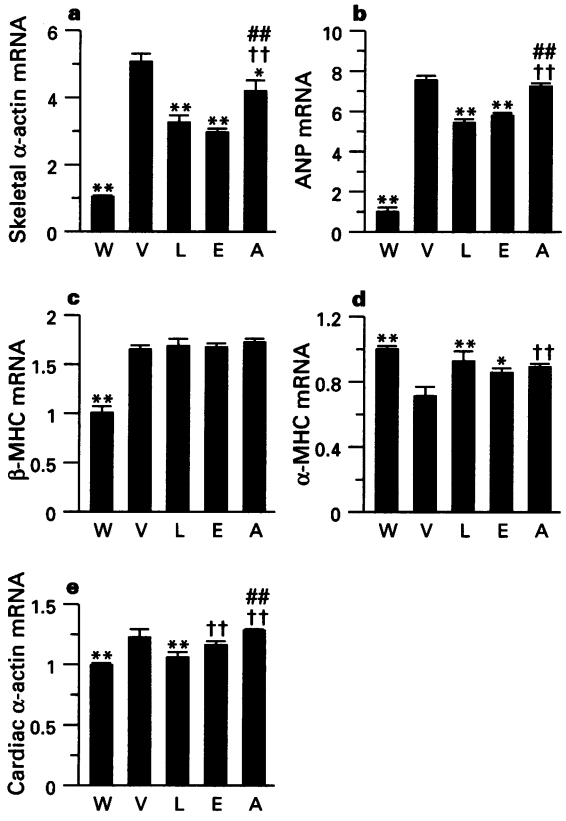

Figure 4 Left ventricular mRNA levels for cardiac phenotype-related genes. In individual samples, each mRNA value was corrected for GAPDH mRNA value. The mean value of mRNA in WKY is represented as 1. Each column represents mean ± s.e.mean of 6 animals per group. Data analysis was carried out by one-way ANOVA and Duncan's multiple range test. $*P<0.05$, $**P<0.01$ compared to V (vehicle-treated SHRSP). $\dagger\dagger P<0.01$ compared to L (losartan-treated SHRSP). $\#\#P<0.01$ compared to E (enalapril-treated SHRSP). For abbreviations, see Figure 2 legend.

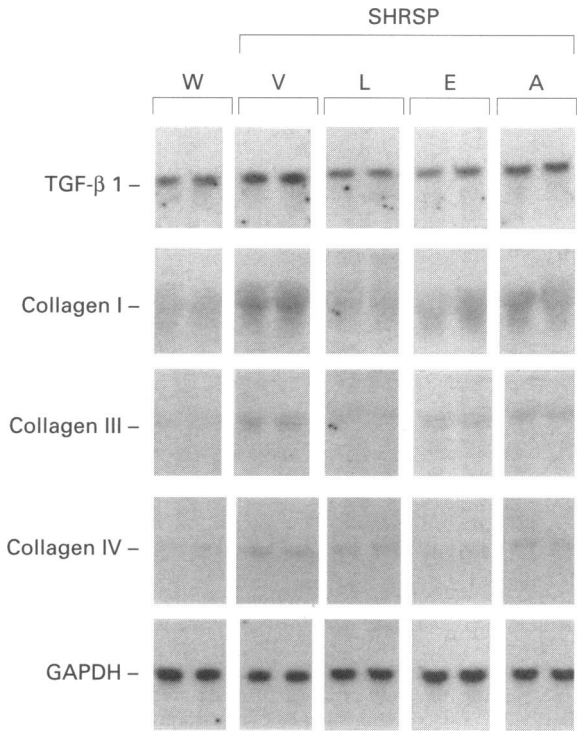

Figure 5 Typical autoradiograms of Northern blot analysis of left ventricular mRNAs for cardiac fibrosis-related genes. W, WKY; V, vehicle-treated SHRSP; L, losartan-treated SHRSP; E, enalapril-treated SHRSP; A, amlodipine-treated SHRSP. The size of mRNA was 2.5 kb for transforming growth factor-β1 (TGF-β1), 4.7 and 5.7 kb for type I collagen (collagen I), 5.9 kb for type III collagen (collagen III), 6.8 kb for type IV collagen (collagen IV), and 1.4 kb for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Autoradiograms of mRNAs from two different animals per group are shown.

Table 2 Ratio of β- to α-myosin heavy chain mRNAs in 24-week-old WKY and SHRSP

Group(n)	*Left ventricle*	*Right ventricle*
WKY (6)	1.00 ± 0.07**	1.00 ± 0.08**
SHRSP		
Vehicle (6)	2.29 ± 0.15	2.57 ± 0.07
Losartan (6)	1.88 ± 0.13*	1.50 ± 0.04**
Enalapril (6)	2.14 ± 0.12	1.32 ± 0.03**
Amlodipine (6)	2.38 ± 0.14†	1.83 ± 0.09**††##

Vehicle, losartan, enalapril and amlodipine indicate SHRSP treatment with each for 8 weeks. Values are the mean ± s.e.mean of 6 animals per group. The mean value of WKY is represented as 1. Statistical significance was determined by one-way ANOVA. $*P<0.05$, $**P<0.01$ compared to vehicle. $\dagger P<0.05$, $\dagger\dagger P<0.01$ compared to losartan. $\#\#P<0.01$ compared to enalapril.

Discussion and conclusions

We have previously reported that left ventricular collagen mRNA levels of 32-week-old SHRSP were increased compared with WKY rats of the same age, and that treatment of SHRSP with an AT_1 receptor antagonist (TCV-116) reduces not only blood pressure but also this increase in left ventricular collagen mRNAs (Kim *et al.*, 1995a). However, in our previous study, the AT_1 receptor antagonist, at the low dose without hypotensive effect, did not decrease left ventricular weight or collagen mRNA levels of SHRSP (Kim *et al.*, 1995a). Therefore, our previous work did not allow us to determine whether or not the suppression of left ventricular hypertrophy and collagen gene expression by the AT_1 receptor antagonist *in vivo* are in part due to the direct action of the AT_1 receptor antagonist, independent of the blood pressure-lowering effect. Furthermore, there is no available report on the effect of anti-hypertensive drugs on myocardial phenotype-related gene expressions of the hypertensive heart.

In the present study, we found the increased expression of two foetal phenotypes of contractile proteins (skeletal α-actin and β-MHC (Nadal-Ginard & Mahdavi, 1989; Parker & Schneider, 1991)) and ANP (another marker of foetal phenotype (Izumo *et al.*, 1988)), and the decreased expression of α-MHC (an adult phenotype of contractile protein (Nadal-Ginard & Mahdavi, 1989; Parker & Schneider, 1991)) in the hypertrophied left ventricle of 24-week-old SHRSP, thereby demonstrating that the transition of myocytes from an adult to a foetal phenotype at the molecular level occurs in the left ventricle of SHRSP of this age. Furthermore, our present study provided evidence that 24-week-old SHRSP had enhanced left ventricular gene expression not only of collagen but also of TGF-β1, a growth factor contributing to interstitial fibrosis via stimulation of collagen production (Eghbali *et al.*, 1991; Border & Ruoslahti, 1992). These findings show that left ventricular hypertrophy of hypertensive rats is associated with the alteration of various gene expressions involved in the modulation of cardiac performance. Thus, investigation of the effects of anti-hypertensive drugs on cardiac gene expression is

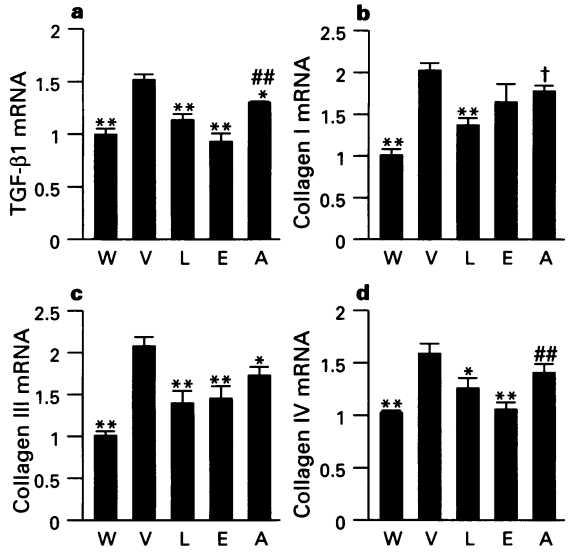

Figure 6 Left ventricular mRNA levels for cardiac fibrosis-related genes. In individual samples, each mRNA value was corrected for GAPDH mRNA value. The mean value of mRNA in WKY is represented as 1. Each column represents mean±s.e.mean of 6 animals per group. $*P<0.05$, $**P<0.01$ compared to V (vehicle-treated SHRSP). $^{\dagger}P<0.05$ compared to L (losartan-treated SHRSP). $\#\#P<0.01$ compared to E (enalapril-treated SHRSP). For abbreviations, see Figure 4 legend.

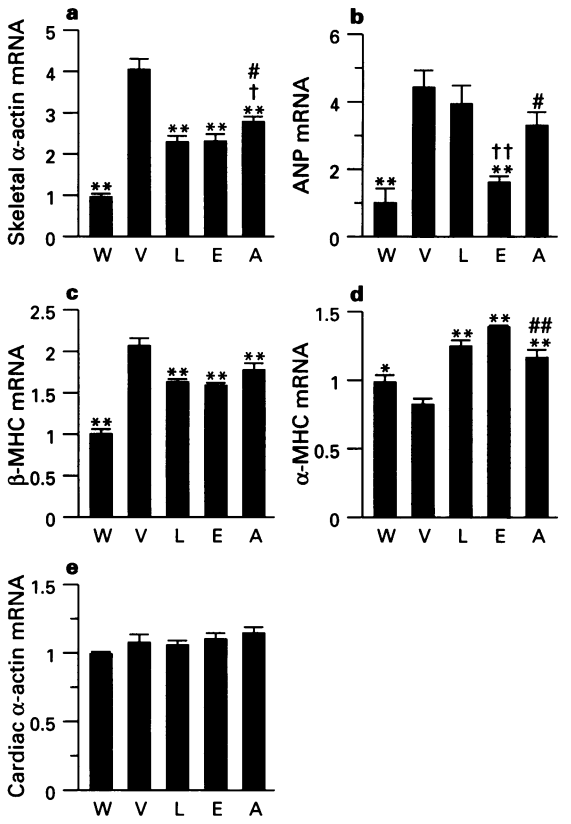

Figure 7 Right ventricular mRNA levels for cardiac phenotype-related genes. Each column represents mean±s.e.mean of 6 animals per group. The mean value of mRNA in WKY is represented as 1. $*P<0.05$, $**P<0.01$ compared to V (vehicle-treated SHRSP). $\dagger P<0.05$, $\dagger\dagger P<0.01$ compared to L (losartan-treated SHRSP). $\#P<0.05$, $\#\#P<0.01$ compared to E (enalapril-treated SHRSP). For abbreviations, see Figure 2 legend.

essential to understand the molecular mechanism of the transition from compensatory left ventricular hypertrophy to left ventricular failure in hypertension.

Normalization of blood pressure of spontaneously hypertensive rats (SHR) with hydralazine (a vasodilator) does not decrease left ventricular weight of SHR, thereby indicating that blood pressure is not the sole factor contributing to cardiac hypertrophy (Sen *et al.*, 1974). It is possible that the calcium channel antagonist may partially produce regression of cardiac hypertrophy directly via inhibition of Ca^{2+} influx, because intracellular calcium has been described as a possible second messenger involved in the process of cell growth (Brostrom *et al.*, 1983). Therefore, it is of great interest to compare the effects of an AT_1 receptor antagonist and a calcium antagonist on cardiac hypertrophy and gene expressions. Amlodipine particularly has not only more potent antihypertensive effects but also a much longer duration of hypotensive action than other popular calcium antagonists such as nifedipine, nitrendipine and verapamil (Fleckenstein *et al.*, 1989), thereby indicating that amlodipine is one of the most powerful and long-acting calcium antagonists. Therefore, in the present study, we used amlodipine as a calcium antagonist to compare with an AT_1 receptor antagonist.

In the present study, losartan, enalapril and amlodipine lowered blood pressure of SHRSP to almost a comparable degree throughout the experiments (Figures 1 and 2a), thereby probably excluding the possibility that the difference in the effect of each drug on cardiac hypertrophy and gene expressions may be due to the difference in hypotensive effect. Of note are the observations that losartan decreased left ventricular weight of SHRSP to a greater extent than amlodipine. Furthermore, as shown in Figures 4 and 6 and Table 2, losartan prevented the shift to foetal phenotype of left ventricular myocytes and suppressed the increased left ventricular collagen gene expressions of SHRSP, while amlodipine failed to alter the gene expression of ANP, α-MHC and collagen types I and IV of SHRSP. Very recently, we have obtained evidence that AII *in vivo* induces cardiac hypertrophy and the shift of cardiac myocytes to a foetal phenotype, independent of blood pressure (Kim *et al.*, 1995b). All these findings, taken together with the evidence for the existence of local renin-an-

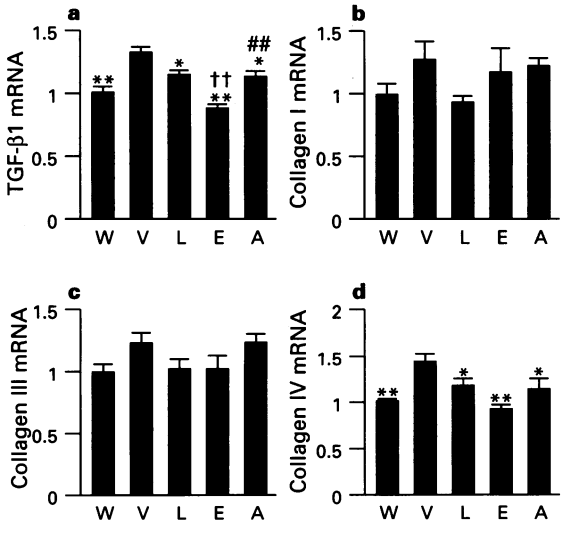

Figure 8 Right ventricular mRNA levels for cardiac fibrosis-related genes. Each column represents mean±s.e.mean of 6 animals per group. The mean value of mRNA in WKY is represented as 1. $*P<0.05$, $**P<0.01$ compared to V (vehicle-treated SHRSP). $\dagger P<0.01$ compared to L (losartan-treated SHRSP). $\#\#P<0.01$ compared to E (enalapril-treated SHRSP). For abbreviations, see Figure 4 legend.

giotensin system in the heart (Lindpainter & Ganten, 1991), demonstrate that the regression of left ventricular hypertrophy and the suppression of abnormal left ventricular gene expressions by AT_1 receptor in SHRSP is at least in part due to its direct action independent of its hypotensive effect.

On the other hand, the effects of losartan on left ventricular hypertrophy and gene expressions of SHRSP were similar to those of enalapril, except for the lack of inhibition of collagen type I expression by enalapril, thereby supporting the idea that the cardioprotective effects of the ACE inhibitor in SHRSP are mainly due to the inhibition of AII generation rather than the inhibition of bradykinin degradation. Although the present study did not allow us to elucidate the reason for the failure of enalapril to suppress left ventricular collagen type I expression, this observation is in good agreement with our previous work (Kim *et al.*, 1995a). It has been reported that an alternative pathway of AII generation, not mediated by ACE, exists in cardiac tissue (Urata *et al.*, 1990). Therefore, it is possible that the lack of suppression of collagen type I may be explained by the inability of enalapril to inhibit the alternative pathway of AII generation. However, further study is needed to verify our assumption.

While the left ventricle ejects blood against a high pressure system, the right ventricle ejects blood against a low pressure system. In addition to the difference in a haemodynamics, *in vitro* data show that significant differences between the right and left ventricular myocardium exist, with respect to mechanical characteristics, such as the time to attain peak total tension and the shortening velocity (Rouleau *et al.*, 1986), and electrophysiological characteristics (Watanabe *et al.*, 1983). Furthermore, the investigations on SHR show that cardiac hypertrophy causes differential effects on right and left ventricular calcium activation (Perreault *et al.*, 1990). However, no comparison has been made between the gene expression of the right and left ventricles in hypertension. In the present study, we also examined the gene expression of the right ventricle of SHRSP to determine whether the differential effects of the AT_1 receptor antagonist and the calcium antagonist on left ventricular gene expressions of SHRSP are also true for the right ventricle. In contrast to the significant increase in left ventricular weight, there was no increase in right ventricular weight of SHRSP, which can be explained by the above mentioned difference in haemodynamics between both ventricles. Interestingly, in spite of no hypertrophic response, the pattern of gene expression in the right ventricle of SHRSP (Figures 7 and 8) was similar to that of the left ventricle, thereby indicating that myocardial transition to a foetal phenotype and the induction of collagen gene expressions occur in a more sensitive manner than myocardial hypertrophic response in hypertension and that the alteration of cardiac gene expressions is independent of the myocyte hypertrophic response. In contrast to the left ventricle, losartan and amlodipine improved these molecular changes in the right ventricle of SHRSP to a similar degree. In addition, unexpectedly, unlike the left ventricle, the increase in right ventricular ANP mRNA levels was suppressed only by enalapril and not by losartan. These findings show that there is a significant difference between the left and right ventricles with respect to the effects of the three drugs on gene expressions, and suggest that the AT_1 receptor may contribute to the gene expression of the left ventricle to a greater degree than that of the right ventricle.

In conclusion, this study shows that the cardiac AT_1 receptor plays a critical role *in vivo* in left ventricular hypertrophy, myocardial phenotypic change and interstitial collagen expression of hypertension. The improvement of hypertension-induced left ventricular hypertrophy and molecular changes induced by the AT_1 receptor antagonist may be at least in part mediated by its direct action, independent of its hypotensive effect.

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