Cardiac Hypertrophy-Related Gene Expression in Spontaneously Hypertensive Rats: Crucial Role of Angiotensin AT$_1$ Receptor

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ABSTRACT—Angiotensin converting-enzyme inhibitors (alacepril and imidapril) or an AT$_1$-receptor antagonist (SC-52458) was administered to 10-week-old spontaneously hypertensive rats (SHR) for 7 days, and cardiac mRNA levels for contractile proteins and atrial natriuretic polypeptide (ANP) were comprehensively measured. The expression of skeletal $\alpha$-actin and ANP was selectively enhanced in the heart of vehicle-treated SHR compared with Wistar-Kyoto rats (WKY), thereby suggesting that the phenotypic modulation of myocytes occurred at the early stage of hypertension. The above-mentioned three drugs similarly suppressed these enhanced gene expressions, nearly to the control levels. In contrast, although the treatment with hydralazine lowered the blood pressure of SHR similarly, hydralazine did not suppress ANP expression at all and only partially suppressed skeletal $\alpha$-actin. Moreover, alacepril did not affect these gene expressions in WKY. Thus, AT$_1$ receptor may be crucial for phenotypic modulation in the heart of SHR.

Keywords: Spontaneously hypertensive rat, Cardiac phenotype, Renin-angiotensin system

The phenotypic modulation of myocytes has been reported to occur during cardiac hypertrophy, which is characterized by re-expression of the fetal phenotype of cardiac contractile proteins and atrial natriuretic polypeptide (ANP). The modulation of these expressions is closely related to myocyte growth induced by various stimuli including mechanical load or hormonal and growth factors, and, therefore, has been considered essential parameters to elucidate the pathophysiology of cardiac hypertrophy (1).

Spontaneously hypertensive rat (SHR) is an excellent model for investigating the pathophysiology of cardiac hypertrophy in human essential hypertension (2). However, the regulation of the above-mentioned cardiac genes in the heart of SHR is poorly understood. In the present study, we examined the short-term effects of renin-angiotensin system (RAS) inhibitors on the cardiac expression of skeletal and cardiac $\alpha$-actin, $\alpha$- and $\beta$-myosin heavy chains (MHC), and ANP in SHR to clarify the role of RAS in these expressions.

Alacepril, 1-[(S)-3-acetylthio-2-methylpropanoyl]-L-prolyl-L-phenylalanine, an ACE inhibitor that has a sulfhydryl (-SH) group in its chemical structure (3), and imidapril, (4S)-1-methyl-3-{(2S)-2-[N-((1S)-1-ethoxycarbonyl-3-phenylpropyl)aminopropionyl]-2-oxo-imidazolidine-4-carboxylic acid hydrochloride without an -SH group (4), were donated by Research Laboratories of Dainippon Pharmaceutical Co., Ltd. (Osaka) and Tanabe Seiyaku Co., Ltd. (Osaka), respectively. SC-52458, 5-[(3,5-dibutyl-1H-1,2,4-triazol-1-yl)methyl]-2-[2-(1H-tetrazol-5-ylphenyl)]pyridine, a non-peptide selective AT$_1$-receptor antagonist (5), were donated by the Searle Research and Development Division of G.D. Searle & Co. (Chicago, IL, USA).

Ten-week-old male SHR were divided into four groups: (I) treatment with 0.5% methylcellulose for 7 days (vehicle group), (II) treatment with alacepril (50 mg/kg/day) for 7 days, (III) treatment with imidapril (10 mg/kg/day) for 7 days, and (IV) treatment with SC-52458 (50 mg/kg/day) for 7 days. For comparison, age-matched WKY were also vehicle-treated. All drugs were orally given once a day by gastric gavage, and blood pressure was measured by the tail-cuff method. In another experiments to clarify whether or not the effects of the above 3 drugs are specific for SHR, WKY were orally treated with alacepril (50 mg/kg/day) for 7 days. Furthermore, to confirm the specific effects of RAS inhibitors, we also treated SHR with hydralazine hydrochloride (Wako Pure Chemical Industries, Ltd., Osaka) (8 mg/100 ml in drinking water, which was consistent with about 9 mg/kg/day)
for 7 days. After the treatment, the animals were killed by decapitation, and the heart of each was rapidly removed and rinsed in ice-cold saline. The left ventricle was separated from the whole heart, immediately frozen in liquid nitrogen, and stored at -80°C until use.

Extraction, agarose gel electrophoresis, and transfer to a nylon membrane of the total RNA from the individual left ventricle were performed as previously described (6). The oligonucleotide probes complementary to the rat skeletal and cardiac α-actin mRNA (7) and rat α- and β-MHC mRNAs (8) were synthesized using a DNA synthesizer. The probes were 5' end-labeled with [32P]-ATP to a specific activity of 6000 Ci/mmol, using a commercially available kit (Takara, Kyoto). Prehybridization and hybridization were performed in 6 × standard saline citrate (SSC) containing 20 mmol NaH2PO4 (pH 7.4), 5 × Denhardt's solution, 0.1% sodium dodecyl sulfate, and 200 µg/ml denatured salmon sperm DNA at 42°C (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). The washing temperature and the sequence of each probe were as follows: 55°C for α-MHC (5'-TTGTGGGATAGCAACACGGA-3'), 53°C for β-MHC (5'-GTCTCAGGGCTTACAGG-3'), 57°C for skeletal α-actin (5'-GCAACCATAGCACGATGGTC-3'), and 51°C for cardiac α-actin (5'-TGACGTGTGTTAAACAAACT-3'); each temperature is consistent with 5°C below the melting temperature. The 825-bp specific cDNA probe for rat ANP was synthesized by the reverse-transcriptase polymerase chain reaction method, from the synthesized oligomer complementary to the sense and antisense strands of the full length cDNA, followed by the sequence analysis using the dideoxy method (9). The 1.3-kb cDNA probe for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was donated by Dr. P. Fort (10). These cDNA probes were labeled and hybridized with the membrane, followed by autoradiography, as previously described (6). The same membrane was rehybridized with another cDNA or oligonucleotide probe after stripping off the hybridized probe by boiling. The autoradiograms were digitized by using an optical scanner and measured for their density using the public domain NIH Image program (6).

As shown in Table 1, the blood pressure of the aloscepiril, imidapril, and SC-52458-treated groups was significantly lower than that of the vehicle-treated group of SHR, and there was no significant difference in the effects among these three drugs. The LV weight of vehicle-treated SHR was larger than that of WKY. Alacepril and imidapril significantly depressed the LV weight of SHR, but SC-52458 did not. Alacepril slightly lowered the blood pressure of WKY (123 ± 3 vs 134 ± 3 mmHg for vehicle treatment) (P < 0.05), but did not affect the LV weight of WKY (229 ± 3 vs 236 ± 3 mg/100 g body weight for vehicle treatment). The blood pressure of hydralazine-treated SHR (146 ± 6 mmHg) was significantly lower than that of the vehicle-treated SHR (201 ± 6 mmHg) (P < 0.001), and the hypotensive effect was similar to those of the 3 RAS inhibitors examined (Table 1). However, hydralazine did not regress the LV hypertrophy of SHR (282 ± 7 vs 296 ± 9 mg/100 g body weight for vehicle treatment).

Figures 1 and 2 show the autoradiograms obtained by northern blot analysis and the results of their densitometric analysis, respectively. The results of densitometric analysis are divided by those of GAPDH, to correct for the difference in loading. In the present study, we demonstrated that the cardiac gene expression of SHR was significantly lower than that of WKY. Alacepril and imidapril significantly depressed the LV weight of SHR, but SC-52458 did not. Alacepril slightly lowered the blood pressure of WKY (123 ± 3 vs 134 ± 3 mmHg for vehicle treatment) (P < 0.05), but did not affect the LV weight of WKY (229 ± 3 vs 236 ± 3 mg/100 g body weight for vehicle treatment). The blood pressure of hydralazine-treated SHR (146 ± 6 mmHg) was significantly lower than that of the vehicle-treated SHR (201 ± 6 mmHg) (P < 0.001), and the hypotensive effect was similar to those of the 3 RAS inhibitors examined (Table 1). However, hydralazine did not regress the LV hypertrophy of SHR (282 ± 7 vs 296 ± 9 mg/100 g body weight for vehicle treatment).

In the present study, we demonstrated that the cardiac gene expression of skeletal α-actin and ANP was selectively enhanced in 11-week-old SHR compared with the age-matched WKY. Interestingly, there was no coconitmate increase in β-MHC gene expression. Ieki et al. reported that in SHR at 38 weeks, but not at 12 or 20 weeks, the cardiac content of β-MHC protein (V3 myosin) was greater than that of WKY (11). Therefore, the increase in β-MHC mRNA may occur in more aged SHR and is not associated with the hypertrophy itself. The enhanced cardiac expression of ANP in 27-week-old SHR was reported by Arai et al. (12), and our results showed that it is also the case in 11-week-old SHR.
Table 1. Body weight, blood pressure and left ventricular weight

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<th>WKY</th>
<th>SHR</th>
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<td>Vehicle</td>
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<td>n</td>
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<tr>
<td>Body weight (g)</td>
<td>301 ± 8</td>
<td>242 ± 6*</td>
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<tr>
<td>Blood pressure (mmHg)</td>
<td>133 ± 2</td>
<td>193 ± 4*</td>
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<td>LV weight (mg/100 g BW)</td>
<td>236 ± 4</td>
<td>279 ± 5*</td>
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Values are the mean ± S.E.M. *P<0.05 vs WKY, †P<0.05 vs SHR (Vehicle).

Fig. 1. Typical autoradiograms of northern blot analysis of cardiac mRNA for skeletal α-actin (SkA), cardiac α-actin (CaA), α-myosin heavy chain (α-MHC), β-MHC, atrial natriuretic polypeptide (ANP) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in WKY and SHR. The following abbreviations were used: W, WKY; V, vehicle-treated SHR; A, SHR treated with alacepril; I, SHR treated with imidapril; S, SHR treated with SC-52458.
(13), these results suggest that the suppression of gene expression may precede the regression of cardiac hypertrophy.

We compared two ACE inhibitors, alacepril and imidapril, with or without the -SH group, respectively, because the -SH group is reported to have cardioprotective effects in some experimental models via acting as a free radical scavenger (14). In our results, both ACE inhibitors showed no difference in the effects on the modulated cardiac gene expression in SHR, thereby suggesting that the -SH group of alacepril (only) has a minor role in the effects on cardiac gene expression. To examine whether the effects of ACE inhibitors on cardiac gene expressions are mediated by activation of the kallikrein-kinin system (15), the effects of an AT1-receptor antagonist, SC-52458, were compared with those of ACE inhibitors, and we found that the effects of SC-52458 on gene expressions are not different from those of ACE inhibitors. Therefore, the effects of ACE inhibitors on cardiac gene expression in SHR seems to be due to the inhibition of Ang II generation. Furthermore, though hydralazine lowered the blood pressure of SHR as much as the 3 types of RAS inhibitors, hydralazine only partially suppressed the enhanced cardiac skeletal α-actin of SHR and did not suppress ANP expression at all. Therefore, the suppressive effects of RAS inhibitors on the cardiac gene expression of SHR may be in part mediated by the direct inhibition of Ang II. This may further be supported by recent evidence that treatment of cultured rat myocytes with Ang II (16) or continuous infusion of a subpressor dose of Ang II to rats in vivo (S. Kim et al., unpublished data) lead to the enhanced gene expression of skeletal α-actin and ANP. In conclusion, Ang II may play an important role in the cardiac phenotypic modulation in vivo.

REFERENCES

8. Gustafson TA, Markham BE and Morkin E: Analysis of


