Original Article

Role of Chymase-Dependent Angiotensin II Formation in Regulating Blood Pressure in Spontaneously Hypertensive Rats

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Vascular smooth muscle cells in spontaneously hypertensive rats (SHR) express angiotensin II-forming chymase (rat vascular chymase [RVCH]), which may contribute to blood pressure regulation. In this study, we studied whether chymase-dependent angiotensin II formation contributes to the regulation of blood pressure in SHR. The systolic blood pressure in 16-week-old Wistar-Kyoto (WKY) rats was 113±9 mmHg, compared to 172±3 mmHg in SHR. Using synthetic substrates for measuring angiotensin-converting enzyme (ACE) and chymase activities, it was found that both ACE and chymase activities in extracts from SHR aortas were significantly higher than in those from WKY rat aortas. Using angiotensin I as a substrate, angiotensin II formation in SHR was found to be significantly higher than that in WKY rats, and its formation was completely suppressed by an ACE inhibitor, but not by a chymase inhibitor. RVCH mRNA expression could not be detected in aorta extracts from either WKY rats or SHR. In carotid arteries isolated from WKY rats and SHR, angiotensin I-induced vasoconstriction was completely suppressed by an ACE inhibitor, but not by a chymase inhibitor. Angiotensin I-induced pressor responses in both WKY rats and SHR were also completely inhibited by an ACE inhibitor, but they were not affected by a chymase inhibitor. In SHR, an ACE inhibitor and an angiotensin II receptor blocker showed equipotent hypotensive effects, but a chymase inhibitor did not have a hypotensive effect. These results indicated that chymase-dependent angiotensin II did not regulate blood pressure in SHR in the present study. (*Hypertens Res* 2005; 28: 457–464)

***Key Words*: angiotensin II, angiotensin-converting enzyme, chymase, vascular smooth muscle cells**

Introduction

Chymase is a chymotrypsin-like serine protease and is known to be present in the secretory granules of mast cells. Chymases have been isolated and their enzymatic characteristics have been studied in rats and humans (*1*–*5*). In general, chymases hydrolyze the C-terminal side of proteins found after aromatic amino acids, such as Phe, Tyr and Trp. Angiotensin I contains two aromatic amino acids, Tyr and Phe, fifth and eighth from the terminus, respectively. While rat chymases, rat mast cell protease (RMCP) I and RMCP II, cleave the bond after the fifth amino acid (Tyr) of angiotensin I to form two inactive fragments, angiotensin-I-(1–4) and angiotensin-I-(5–10), human chymase cleaves the bond after the eighth (Phe) to yield angiotensin II (*3*, *6*). In fact, in isolated human

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vessels, the vascular contraction induced by angiotensin I is partially suppressed by an angiotensin-converting enzyme (ACE) inhibitor, and the remaining contraction is then completely suppressed by a chymase inhibitor. On the other hand, in isolated rat vessels of Sprague-Dawley (SD) rats, the vascular contraction is completely suppressed only by an ACE inhibitor (*7*). These findings suggest that human vessels contain ACE- and chymase-dependent angiotensin II pathways, but that rat vessels contain only the ACE-dependent angiotensin II-forming pathway.

On the other hand, Akasu *et al*. (*8*) reported that chymase-dependent angiotensin II formation was more dominant than ACE-dependent angiotensin II formation in aorta extracts of SD rats. Recently, Guo *et al*. (*9*) purified a novel rat chymase, rat vascular chymase (RVCH), from vascular smooth muscle cells (SMCs) that could convert angiotensin I to angiotensin II, and they found that the RVCH mRNA levels in the vascular SMCs of spontaneously hypertensive rats (SHR) were significantly higher than those of SD rats. Furthermore, the conditional and targeted overexpression of RVCH in vascular SMCs was found to cause hypertension in transgenic mice (*10*). Therefore, RVCH might be involved in the pathophysiology of hypertension in SHR.

In the present study, to clarify whether chymase was involved in the regulation of blood pressure in SHR, we studied the involvement of chymase in the angiotensin II-forming ability of vascular extracts, in the angiotensin I-induced vascular contraction of isolated vessels, and in the angiotensin I-induced pressor response of Wistar-Kyoto (WKY) rats and SHR. Furthermore, we compared the hypotensive effects of an ACE inhibitor, a chymase inhibitor, and an angiotensin II receptor blocker (ARB) in SHR.

Methods

Animals

Sixteen-week-old male WKY rats and SHR were obtained from Japan SLC Inc. (Shizuoka, Japan). All rats were housed at room temperature (23–26°C) with a 12-h light-dark cycle and had free access to standard food (F-2; Funahashi Co., Tokyo, Japan) and water. The experimental procedures for animals were in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College, Takatsuki, Japan).

Rat Vascular Tissue

The rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). The aorta was removed for experiments on rat vascular tissue extracts, and the carotid arteries were removed for experiments dealing with vasoconstriction of isolated arteries. When sufficient anesthetization had been achieved, the aorta was immediately removed, frozen in liquid nitrogen, and stored at −80°C for enzyme assay and mRNA level measurements. The carotid arteries were transported in ice-cooled Tyrode's solution consisting of 137 mmol/l NaCl, 2.7 mmol/l KCl, 1.8 mmol/l $CaCl_2$, 1.1 mmol/l $MgCl_2$, 0.42 mmol/l NaH_2PO_4, 12 mmol/l $NaHCO_3$, and 5.7 mmol/l glucose at a pH of 7.4, and they were stored at 4°C while awaiting evaluation of angiotensin I-induced vasoconstriction.

Extraction from Rat Vascular Tissue

The aortas were homogenized in 10 volumes (w/v) of 20 mmol/l phosphate buffer, pH 7.4 (*11*). The homogenate was centrifuged at 20,000 rpm for 30 min. The supernatant was discarded, and the pellet was re-suspended and homogenized in 5 volumes (w/v) of 10 mmol/l phosphate buffer, pH 7.4, containing 2 mol/l KCl and 0.1% nonidet P-40. The homogenate was centrifuged at 20,000 rpm for 30 min, and the supernatant was used to measure the ACE and chymase activities using specific synthetic substrates. ACE inhibitor and chymase inhibitor effects on angiotensin II-forming activity were also studied.

Measurements of ACE and Chymase Activities

ACE activities were measured by incubating the plasma or the tissue extracts for 30 min at 37°C with 5 mmol/l hippuril-His-Leu (Peptide Institute, Minoh, Japan) as the substrate when measuring ACE activity in 100 mmol/l phosphate buffer, pH 8.3, containing 800 mmol/l NaCl (*12*). The enzyme reaction was terminated by the addition of 3% metaphosphoric acid (w/v), and the resulting mixture was placed in ice water for 10 min. After centrifugation of the mixture at 15,000 rpm for 5 min, we applied 50 μl of the supernatant to an ODS reversed-phase column (4.6 mm × 25 cm; Tosoh, Tokyo, Japan), which had been equilibrated with 10 mmol/l KH_2PO_4 and CH_3OH (1:1, pH 3.0), and eluted it with the same solution at a rate of 0.3 ml/min. Hippuric acid was detected by ultraviolet absorbance at 228 nm. One unit of ACE activity was defined as the amount of enzyme that cleaved 1 μmol hippuric acid/min.

Chymase activity was measured by incubating the tissue extracts for 30 min at 37°C with 5 mmol/l Suc-Ala-Ala-Pro-Phe-4-methylcoumaryl-7-amide (Peptide Institute) as a substrate for the measurement of chymase activity in 100 mmol/l Tris-HCl buffer, pH 8.5, containing 200 mmol/l NaCl (*13*). The enzyme reaction was terminated by the addition of 3% metaphosphoric acid (w/v), and the reaction mixture was placed in ice water for 10 min. After centrifugation of the reaction mixture at 15,000 rpm for 5 min, 7-amino-4-methylcoumarin (AMC) was measured by fluorophotometric determination (excitation, 380 nm; emission, 460 nm). One unit of chymase activity was defined as the amount of enzyme that cleaved 1 μmol AMC/min.

Protein concentration was assayed with BCA Protein Assay Reagents (Pierce, Rockford, USA) using bovine serum albumin as a standard.

Conversion of Angiotensin I in Extract from Vascular Tissues

Aliquots of the extract were incubated for 30 min at 37°C with 5 mmol/l angiotensin I in 10 mmol/l phosphate buffer, pH 7.4, containing 150 mmol/l NaCl (*14*, *15*). To study the effects of various drugs, an ACE inhibitor, trandolaprilat (1 μmol/l), or a chymase inhibitor, NK3201 (1 μmol/l), was added, and preincubation was conducted for 10 min at 37°C, followed by incubation for 30 min at 37°C with 5 mmol/l angiotensin I in 10 mmol/l phosphate buffer, pH 7.4, containing 150 mmol/l NaCl. The reaction was terminated by the addition of 15% trichloroacetic acid (w/v), followed by centrifuging at 20,000 × *g* for 10 min. The supernatant was applied to an ODS reversed-phase column (4.6 mm × 25 cm; Tosoh), which was pre-equilibrated with 20% acetonitrile containing 0.1% trifluoroacetic acid. The column was first eluted with the same eluent for 25 min at a flow rate of 1.0 ml/min, then with a linear gradient of acetonitrile (20–35%) for 5 min, and finally with a linear gradient of acetonitrile (35–70%) for 5 min. Angiotensin II was detected by ultraviolet absorbance at 218 nm. One unit of angiotensin II-forming activity was defined as the amount of enzyme that formed 1 μmol angiotensin II/min.

Reverse Transcription (RT)–Polymerase Chain Reaction (PCR)

The total RNA of the aorta was extracted using Trizol reagent (Life Technologies, Rockville, USA) and dissolved in 0.1% diethyl pyrocarbonate-treated water. RT to cDNA was accomplished by analyzing 5 μg of the total RNA sample with SuperScript II reverse transcriptase and oligo(dT)$_{12–18}$ primer (Invitrogen, Carlsbad, USA). The reaction was carried out in the presence of first-strand buffer, 1 mmol/l dNTPs and 20 mol/l dithiothreitol, at 42°C for 50 min. The PCR mixture contained 1 μl of the cDNA reaction mixture, 20 pmol/l primers, PCR buffer, 0.4 mmol/l dNTPs, and 2.5 U *Taq* polymerase. The reaction was carried out with a RoboCycler (Stratagene, La Jolla, USA). The sequences of the oligonucleotide primers for PCR were as follows: RVCH sense primer, 5′-GAGGCCTGTAAAATCTATAGAC-3′, and antisense primer, 5′-TGTGTATCTTTGAGAGCCTCAA-3′, were used for the amplification of RVCH (*9*); RMCP I sense primer, 5′-ACCACTGAGAGAGGTTACAAGGCCAC-3′, and antisense primer, 5′-GTTATAATGAAAATAGTTTTTACAG GCCTC-3′, were used for the amplification of RMCP I (*16*); RMCP II sense primer, 5′-GCCTGTGTGGACTACAGGTA-3′, and antisense primer, 5′-TCAGGCTTTTCAGCTAC TTG-3′, were used for the amplification of RMCP II (*17*); and β-actin sense primer, 5′-CCAAGCCAACCGCGAGAAGAT GAC-3′, and antisense primer, 5′-AGGGTACATGGTGGT GCCGCCAGAC-3′, were used for the amplification of β-actin for the calibration of sample loading (*18*). The PCR products were separated by electrophoresis on 2% agarose gel stained with ethidium bromide and the samples were then visualized by ultraviolet transillumination.

To obtain the band for a positive reference of RVCH expression, we carried out RT-PCR using hypertrophied pulmonary tissues harvested from SD rats in which pulmonary hypertension had been induced by an injection of the toxin monocrotaline (*16*).

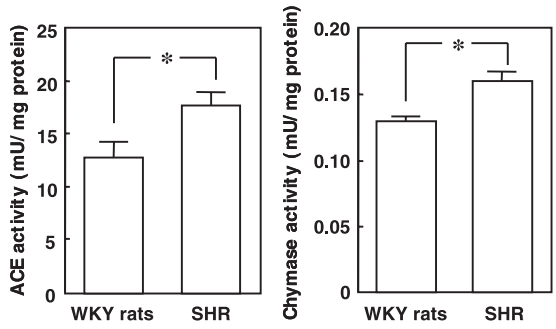

Fig. 1. *ACE and chymase activities in extracts from the aortas in WKY rats and SHR. Bars indicate the means±SEM (*n = *8). *p < *0.05* vs. *WKY rats.*

Angiotensin I-Induced Vasoconstriction in Isolated Rat Arteries

The rat carotid artery was cut into helical strips, 10 mm in length and 1.0 mm in width (*7*). Each artery strip was placed on a myograph with a resting tension of 1.0 g. The bathing medium was Tyrode's solution consisting of 137 mmol/l NaCl, 2.7 mmol/l KCl, 1.8 mmol/l $CaCl_2$, 1.1 mmol/l $MgCl_2$, 0.42 mmol/l NaH_2PO_4, 12 mmol/l $NaHCO_3$, and 5.7 mmol/l glucose, pH 7.4. The medium was maintained at 37°C and bubbled continuously with 5% CO_2 in oxygen. The strip was equilibrated for 2 h before the experiments dealing with the angiotensin I-induced vascular contractile response were done. At that time, vasoconstriction was obtained with 50 mmol/l KCl, and then the bathing medium was washed out. The medium was washed out 3 times for 15 min each time with fresh Tyrode's solution and equilibrated for 30 min. Next, angiotensin I (100 nmol/l) was added to the bathing medium. The steps described above for the angiotensin I response were repeated, and the second angiotensin I response was regarded as the control response for angiotensin I. After this response was obtained, the medium was washed out 2 times for 15 min each time with Tyrode's solution. An ACE inhibitor, trandolaprilat (1 μmol/l), or a chymase inhibitor, NK3201 (1 μmol/l), was added, and preincubation was conducted for 20 min. This was followed by the addition of angiotensin I.

Pressor Response of Angiotensin I

To study the pressor response, the rats were anesthetized with

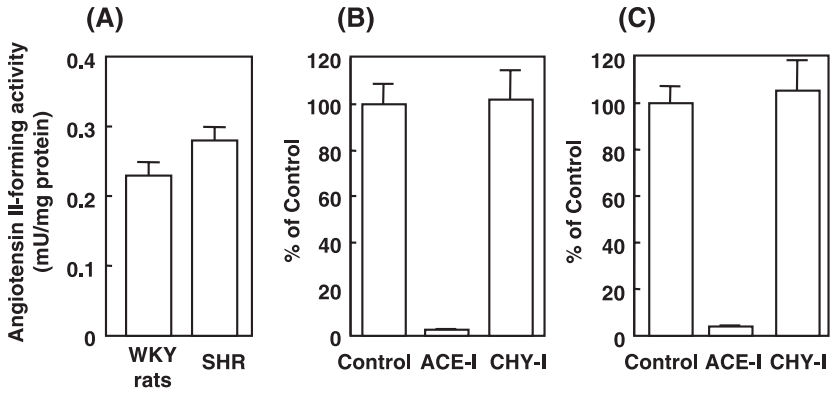

Fig. 2. *Angiotensin II-forming activities in extracts from the aortas in WKY rats and SHR (A). Effects of an ACE inhibitor (ACE-I) and a chymase inhibitor (CHY-I) on angiotensin II-forming activities in WKY rats (B) and SHR (C), when the angiotensin II-forming activity in the absence of protease inhibitors was regarded as 100% (control). Bars indicate the means±SEM (*n = *8).*

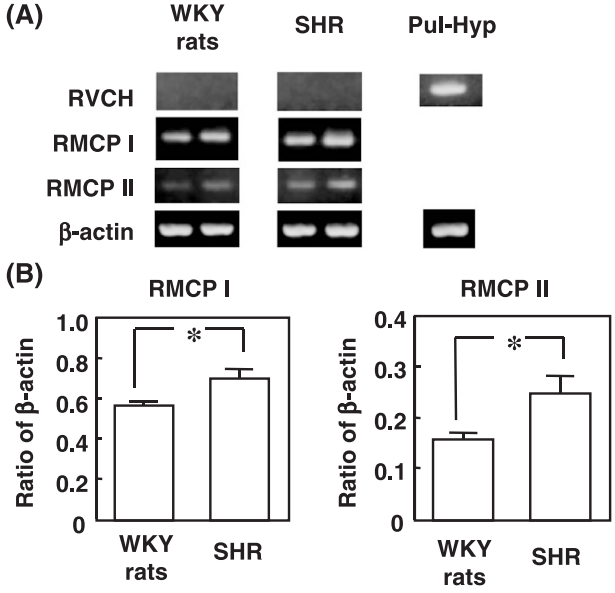

Fig. 3. *RT-PCR products of RVCH, RMCP I and RMCP II in extracts from the aortas in WKY rats and SHR, and from the lungs in monocrotaline-induced pulmonary hypertensive rats (Pul-Hyp). Ratio of RMCP I or RMCP II to β-actin mRNA in the extracts from the aortas in WKY rats and SHR (B). Bars indicate the means±SEM (each group,* n = *6).* *p < *0.05* vs. *WKY rats (*n = *6).*

sodium pentobarbital (50 mg/kg i.p.). A PE-10 catheter (Clay Adams, Parsippany, USA) was inserted into the left femoral artery and was connected to a pressure transducer (TP-200T; Nihon Kohden, Tokyo, Japan), to measure the systolic blood pressure (SBP). The other catheter was inserted into the left femoral vein. After the SBP was stabilized, angiotensin I (100 ng/kg) was administered *via* the catheter. The intravenous administration of angiotensin I was repeated until constant pressor responses were obtained. For the studies dealing with the effects of trandolaprilat (3 mg/kg) or NK3201 (10 mg/kg), the drugs were administered 10 min before the injection of angiotensin I, and the pressor response was then measured.

Effects of Agents on Blood Pressure in SHR

The 24-h time course of changes in SBP after a single oral dose of trandolapril (10 mg/kg), NK3201 (10 mg/kg) or candesartan cilexetil (10 mg/kg) was monitored by tail-cuff plethysmography (BP-98; Softron Co., Tokyo, Japan). Rats were placed in a restraining cage and warmed for 10 min before taking measurements. Three consistent measurements of SBP were averaged to obtain a reading for each rat.

Statistical Methods

Data are expressed as the mean±SEM. When two groups were compared, differences were assessed by the unpaired Student' *t*-test. Other statistical analyses were performed using a parametric test with Fisher's Protected Least Significant Difference. Differences were considered significantly only when the *p* value was <0.05.

Results

Body Weight and SBP

There was no statistically significant difference in the average weights of the WKY rats (358±10 g) and the SHR (364±5 g). A significant difference in SBP was observed between the WKY rats (113±9 mmHg) and the SHR (172±3 mmHg).

ACE and Chymase Activities in Vascular Extract

A significant difference was observed in the ACE activity of

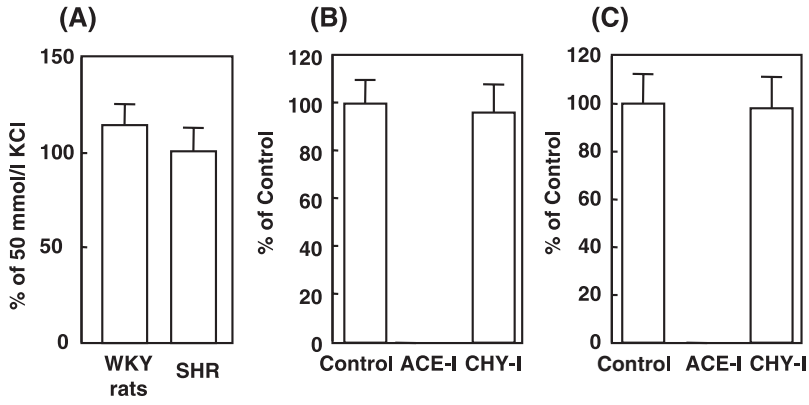

Fig. 4. *Angiotensin I-induced vasoconstrictions in isolated carotid arteries in WKY rats and SHR (A). Effects of an ACE inhibitor (ACE-I) and a chymase inhibitor (CHY-I) in angiotensin I-induced vasoconstrictions in WKY rats (B) and SHR (C), when the angiotensin I-induced vasoconstriction in the absence of protease inhibitors was regarded as 100% (control). Bars indicate the means ± SEM (*n = *6).*

aortic extracts taken from WKY rats (12.8±1.5 mU/mg protein) and SHR (17.8±1.2 mU/mg protein) (Fig. 1). Similarly, a significant difference was also observed in the chymase activity of aortic extracts taken from WKY rats (0.13±0.004 mU/mg protein) and SHR (0.16±0.008 mU/mg protein) (Fig. 1).

Angiotensin II-Forming Activity in Vascular Extract

While angiotensin II-forming activity in the extract taken from the aortas of WKY rats (0.23±0.02 mU/mg protein) tended to be lower than that in the extract taken from the aortas of SHR (0.28±0.02 mU/mg protein) (Fig. 2), this difference was not statistically significant.

In the presence of an ACE inhibitor, angiotensin II-forming activities were almost abolished in the extracts from the aortas of both WKY rats and SHR (Fig. 2). However, the angiotensin II-forming activities were not affected by the presence of a chymase inhibitor (Fig. 2).

mRNA Levels of Chymases and ACE

RT-PCR results are shown in Fig. 3. RVCH mRNA expression was not detected in the extracts taken from the aortas of WKY rats and SHR, but the band used as a positive reference of RVCH, which was obtained from the pulmonary tissues of monocrotaline-induced pulmonary hypertensive rats, was clearly detected (Fig. 3). The mRNA expressions of RMCP I and RMCP II were significantly higher in the SHR than in the WKY rats (Fig. 3).

Angiotensin I-Induced Vasoconstriction of Isolated Arteries

In the isolated rat artery, angiotensin I-induced vasoconstriction in SHR was not significantly different from that seen in WKY rats, although the angiotensin I-induced vasoconstriction tended to be weaker in SHR (Fig. 4). In these two strains, angiotensin I-induced vasoconstriction was completely suppressed in the presence of an ACE inhibitor, but vasoconstriction was not affected by the presence of a chymase inhibitor (Fig. 4).

Angiotensin I-Induced Pressor Response

Under anesthesia, the SBP in WKY rats was 110±9 mmHg, while in SHR the SBP was 140±6 mmHg. The maximum change in SBP after the injection of angiotensin I in WKY rats was 46±4 mmHg, while in SHR it was 56±8 mmHg; this difference was not significant (Fig. 5). After treatment with a chymase inhibitor, the maximum change in SBP after injection of angiotensin I in WKY rats was 48±6 mmHg, while in SHR it was 57±9 mmHg. In both WKY rats and SHR, the maximum change in the SBP was not different from that noted following pretreatment with a chymase inhibitor (Fig. 5). On the other hand, after treatment with an ACE inhibitor, the angiotensin I-induced pressor responses in both WKY rats and SHR were completely abolished (Fig. 5).

Depressor Effects of ACE inhibitor, Chymase Inhibitor, and ARB in SHR

The SBP in SHR was significantly reduced even 24 h after the oral administration of an ACE inhibitor and an ARB. The maximum depressor effects of these agents were almost equal 4 h after oral administration (Fig. 6). However, the SBP was not significantly affected at 2, 4, 8, and 24 h after the administration of a chymase inhibitor (Fig. 6).

Discussion

In rat vascular tissues, RMCP I and RMCP II are likely

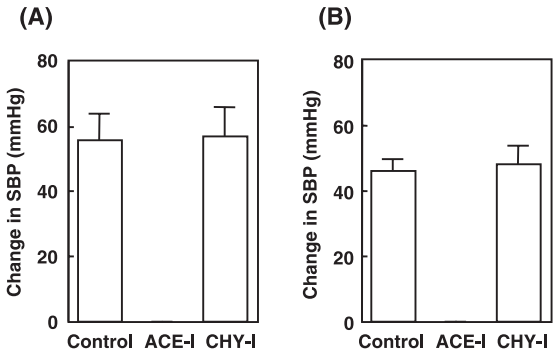

Fig. 5. *Changes in SBP after intravenous injection of angiotensin I in the absence of protease inhibitors (control) and in the presence of an ACE inhibitor (ACE-I) or a chymase inhibitor (CHY-I) in WKY rats (A) and SHR (B). Bars indicate the means±SEM (*n = *6).*

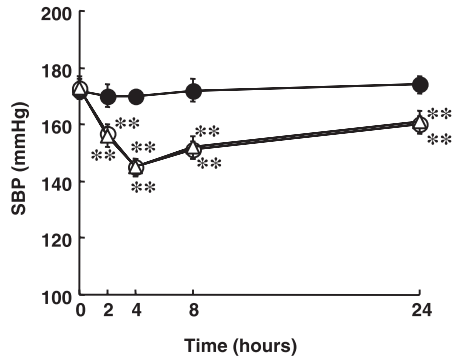

Fig. 6. *Effects of an ACE inhibitor (○), an ARB (△), and a chymase inhibitor (●) on SBP 2, 4, 8 and 24 h after administration. Each point is the mean±SEM (*n = *6).* **p < *0.01* vs. *pre-injection of an ACE inhibitor or an ARB.*

located in the mast cells found in vascular tissues, and RVCH might be expressed by vascular SMCs. In the present study, however, mRNA expression of RMCP I and RMCP II was detected in both WKY rats and SHR, but RVCH mRNA was not detected. Guo *et al*. (*9*) reported that RVCH mRNA expression in vascular SMCs obtained from the aortas of 8-week-old SHR was higher than in age-matched SD rats. However, they could not detect RVCH mRNA expression in the whole tissues of the aorta, which was also our experience in the present study, although we used 16-week-old SHR as the hypertensive model. This finding suggests that angiotensin II-forming chymase RVCH is very weakly expressed in the aorta of not only WKY rats but also SHR. In fact, angiotensin II-forming activity tended to be higher in the extracts taken from the aortas of SHR than in those taken from the aortas of WKY rats. Nevertheless, angiotensin II-forming activity was almost completely suppressed by treatment with an ACE inhibitor, but not by treatment with a chymase inhibitor. Furthermore, an ACE inhibitor completely suppressed the angiotensin I-induced pressor response in WKY rats and in SHR. An ACE inhibitor and an angiotensin II receptor blocker showed equipotent hypotensive effects in SHR, but a chymase inhibitor did not. These findings suggest that chymase-dependent angiotensin II has little involvement in the regulation of blood pressure in SHR.

In the present study, we used the synthetic substrate Suc-Ala-Ala-Pro-Phe-MCA, which is useful for measuring chymotrypsin-like enzyme activity, including chymase activity (*13*). The activities in the extract taken from the vascular tissues of WKY rats and SHR were completely inhibited by treatment with a specific chymase inhibitor, NK3201. This suggests that these activities were almost completely dependent on chymases, RMCP I, RMCP II, and RVCH, found in the vascular tissues. However, RVCH mRNA expression was not detected in the aorta of either rat strain, although its expression was detected in the pulmonary tissues of rats with pulmonary hypertension, as has been previously reported (*9*). On the other hand, mRNA expression of RMCP I and RMCP II in the aorta was significantly higher in SHR than in WKY rats. Therefore, the high level of chymase activity in the vascular tissues of SHR is thought to depend on an increase in the mRNA expressions of RMCP I and RMCP II, but not of RVCH. Both RMCP I and RMCP II cleave the bond found after Tyr-5 of angiotensin I to form inactive fragments, angiotensin-I-(1–4) and angiotensin-I-(5–10) (*6*). In the present study, angiotensin-I-(5–10) formation was significantly higher in SHR than in WKY rats, and this formation was completely suppressed by treatment with a chymase inhibitor (data not shown). Therefore, when angiotensin I is the substrate, chymase activity in vascular tissues of SHR may play a role in degrading angiotensin I to inactive fragments rather than forming angiotensin II.

In the present study, not only vascular ACE activity but also angiotensin II-forming activity was higher in SHR than in WKY rats. An increase of ACE in the vascular tissues of SHR may play an important role in increasing vascular angiotensin II-forming ability. The significance of vascular ACE expression in the regulation of blood pressure in SHR has been reported by previous papers (*19*, *20*). Although angiotensin II-forming chymase RVCH was not detected, non-angiotensin II-forming chymases RMCP I and RMCP II were increased in the vascular tissues of SHR. Shiota *et al*. (*21*) reported that RMCP I mRNA expression in SHR hearts was significantly higher than in WKY rat hearts. RMCP I may be involved in cardiovascular remodeling *via* the degradation of fibronectin (*22*, *23*) and by the activation of transforming growth factor-β (*24*, *25*). On the other hand, RMCP II may contribute to an increase of vascular permeability (*26*). Therefore, the upregulated chymases, RMCP I and RMCP II, that are found in SHR vascular tissues may be involved in remodeling vascular structures rather than in regulating blood pressure.

In the present study, when we used 10 mg/kg of NK3201 as

a chymase inhibitor we did not find that chymase contributed to the regulation of blood pressure in SHR. NK3201 is synthesized as an orally active chymase inhibitor (Nippon Kayaku Co. Ltd., Tokyo, Japan) with a known chemical structure (*27*). NK3201 inhibits human chymase with an IC_{50} of 2.5 nmol/l and inhibits RMCP I with an IC_{50} of 12 nmol/l (*27*). In rats, NK3201 is absorbed rapidly into the plasma after the oral administration of 1 mg/kg of NK3201 (*28*). NK3201 has a concentration of approximately 100 nmol/l in plasma 24 h after oral administration. Furthermore, NK3201 is detected 24 h after oral administration at concentrations over 10 nmol/l in the heart, aorta, lung, liver, kidney, muscle, and skin, but not in the brain (*28*). In fact, the oral administration of 10 mg/kg of NK3201 significantly reduces renal injury in Dahl salt-sensitive hypertensive rats (*29*). In the present study, although the oral administration of 10 mg/kg of NK3201 was thought to be sufficient to prevent chymase *in vivo*, NK3201 failed to affect the blood pressure in SHR. In transgenic mice, Guo *et al*. (*9*) reported that the conditional and targeted overexpression of RVCH in vascular SMCs caused hypertension. A transgenic mouse model carrying the human chymase gene was observed to have elevated blood pressure, and its hypertension was abolished by an ARB (*10*). Koga *et al*. (*30*) demonstrated that *in vivo* expression of human chymase in mice caused mild hypertension with left ventricular hypertrophy and also chronic inflammatory changes. Moreover, they reported that chymase-dependent angiotensin II formation was also observed in the wild-type littermates. In fact, a study of mouse aortas found that the dominant chymase mRNAs were mouse chymase 4 and 5, both of which could form angiotensin II from angiotensin I, and that these chymases contributed to the regulation of blood pressure *via* angiotensin II formation (*31*). These reports suggest that chymase-dependent angiotensin II may be partially involved in the regulation of blood pressure. However, other studies reported that the chymase gene was unlikely to have influenced blood pressure levels in a Japanese population (*32*, *33*). In the present study, we evaluated only the typical hypertensive SHR model, and further studies of the relationship between angiotensin II-forming chymase and the hypertensive pathophysiology in other hypertensive models and in human hypertensives are needed.

In conclusion, the angiotensin II-forming ability demonstrated by chymases found in the aorta was not detected in either SHR or WKY rats, and a specific chymase inhibitor had no hypotensive effect on the SBP of SHR. Our data suggest that chymase-dependent angiotensin II formation in vascular tissues might not be involved in the hypertensive mechanism of SHR.

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