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Intrarenal angiotensin converting enzyme inhibition in spontaneously hypertensive rats

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Abstract

We examined the hypotensive effect of enalapril in relation to the local renin–angiotensin system of the kidney in spontaneously hypertensive rats (SHR). Oral administration of enalapril for 7 days decreased mean arterial blood pressure and renal tissue angiotensin II concentration without affecting plasma angiotensin II concentration in SHR. The enalapril treatment did not affect maximum binding of angiotensin II to renal tubules and glomeruli in SHR. In normotensive Wistar–Kyoto rats, no significant changes in mean arterial blood pressure, renal and plasma angiotensin levels were observed with enalapril treatment. Direct infusion of enalapril into the renal medullary interstitium decreased mean arterial blood pressure in association with the reduction of renal tissue angiotensin II concentration without changes in plasma angiotensin II concentration in SHR. These observations suggest that the inhibition of angiotensin conversion in the kidney is important for the hypotensive action of enalapril.

Keywords: Angiotensin II; Spontaneously hypertensive rats (SHR); Hypertension; Kidney

1. Introduction

Angiotensin converting enzyme inhibitors are valuable antihypertensive agents, but their hypotensive effects cannot be simply attributed to the suppression of the circulating angiotensin II level. These drugs have been reported to show only transient reduction of the circulating angiotensin II, with levels returning toward normal, despite a sustained hypotension (Mento et al., 1989). One of the explanations for the discrepancy between circulating angiotensin II level and blood pressure may be attribute to the role of local renin–angiotensin system on blood pressure regulation. Molecular biological studies have shown the presence of components of the renin–angiotensin system and their distinct regulation in many different tissues (Dzau et al., 1988). In the presence of angiotensin converting enzyme, angiotensin II is generated and acts locally, thus not requiring any systemic delivery of angiotensin II (Dzau, 1984).

The local renin–angiotensin system of the kidney is thought to have an important role in the regulation of renal functions and blood pressure (Zimmerman and Dunham, 1997). Bilateral nephrectomy has been reported to abolish the antihypertensive response of angiotensin converting enzyme inhibitor in spontaneously hypertensive rats (SHR) (Inagami et al., 1991). These observations suggest that kidney may be an important site of action of angiotensin converting enzyme inhibitors.

In the present study, to clarify the role of local renin–angiotensin system of the kidney in the hypotensive effect of angiotensin converting enzyme inhibitor in SHR, first, we examined the effects of oral administration of enalapril on blood pressure and the renal levels of angiotensin peptides and angiotensin receptor. Second, we investigated the effects of local inhibition of renal renin–angiotensin system on blood pressure by intrarenal infusion of enalapril.

2. Materials and methods

2.1. Animals

Male Wistar–Kyoto rats (WKY) and SHR were purchased from Funabashi Farm (Chiba, Japan). Rats received a standard rat chow and tap water freely and were kept in a room at a temperature of 23°C. At the beginning of enalapril treatment, rats were 14 weeks of age.

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2.2. Protocol A: oral administration of enalapril

Effects of oral enalapril administration on plasma and renal angiotensins were examined in 13 WKY and 15 SHR. Before the enalapril treatment, systolic blood pressure was measured in all rats by the tail-cuff method using an electrosphygmomanometer (Tokai Irika, Tokyo, Japan). Rats were then kept in individual metabolic cages and were measured for water intake every day. Enalapril was dissolved in 30 mg/l in drinking water and was administered to eight WKY and eight SHR. At fourth or fifth day of enalapril treatment, a catheter for the measurement of blood pressure was inserted into the femoral artery under pentobarbital anesthesia (50 mg/kg, i.p.). At the seventh day of enalapril treatment, the catheter was connected to a pressure transducer (model TP-200T, Nihon-Kohden, Tokyo, Japan) and blood pressure and heart rate were measured in conscious unrestrained state. Then, rats were killed by decapitation without prior anesthesia. Trunk blood was collected for the measurement of plasma angiotensin peptides, and kidneys were isolated for the measurement of renal angiotensin levels and for binding assay.

2.3. Protocol B: renal medullary interstitial infusion of enalapril

The effects of renal infusion of enalapril (0.3 mg kg^{-1} day^{-1}, $n = 5$; 3 mg kg^{-1} day^{-1}, $n = 6$) or vehicle (saline, $n = 6$) on blood pressure and renal angiotensins were examined in SHR. To eliminate any compensatory response from the contralateral kidney, the left kidney was removed under pentobarbital anesthesia (50 mg/kg, i.p.). Rats were allowed for 1 to 2 weeks for recovery from this surgery.

Renal medullary interstitial catheter was implanted according to the method reported by Lu et al. (1994). The right kidney was exposed via a flank incision, and the PE-10 tubing was inserted through renal capsule to a depth of 4–5 mm to reach the base of the papilla. The catheter was tunneled subcutaneously to the back of neck and connected with an osmotic minipump (model 2ML1; Alza, Palo Alto, CA) containing enalapril maleate. The osmotic minipump was implanted subcutaneously at dorsum of the neck. A catheter for the measurement of blood pressure was inserted into the femoral artery. Blood pressure and heart rate were measured daily throughout the enalapril treatment. After 7 days of enalapril treatment, the kidney and blood were collected by the same procedure as protocol A.

2.4. Extraction of angiotensin peptides from rat plasma and kidney

Trunk blood (2–3 ml) was rapidly collected into a chilled (4°C) tube containing 0.5 ml inhibitor solution (42 mM 1,10-phenanthroline, 100 mM ethylenediaminetetraacetate $2NH_4$, 1.7 mg/ml neomycin sulfate, 2 mg/ml pepstatin, and 1.7% ethanol). The blood was centrifuged and the plasma (1 ml) was immediately applied to an octadecasilyl-silica cartridge (Amersham, Buckinghamshire, England), and washed with 10 ml of 0.1% trifluoroacetic acid. The angiotensin peptides were eluted with 6 ml methanol: water: trifluoroacetic acid (80:19:1, v/v/v). The eluate was dried in a vacuum centrifuge and stored at 4°C until high-performance liquid chromatography (HPLC) analysis.

The right kidney was weighed, and homogenized in 20 ml of solution containing 4 M guanidine thiocyanate and 1% (v/v) trifluoroacetic acid. Tissue homogenates were sonicated briefly and then centrifuged at $5000 \times g$ for 20 min. A 2.5 ml of the supernatant from each homogenate was extracted, evaporated, and stored as described for plasma.

2.5. HPLC analysis of angiotensin peptides

The extracted samples were dissolved in 50 mM phosphate buffer, pH 7.4 (solvent A): acetonitrile (solvent B) (80:20), and centrifuged at $3000 \times g$ for 10 min. The supernatant was injected into the HPLC system (pump-model 510, tunable absorbance detector-model 484, automated gradient controller-model 680, column-μ-bondasphere 5μ C18-100 Å (3.9 × 150 mm); Waters). Peptides were eluted using a number 8 gradient curve from 20 to 50% solvent B over 18 min; flow rate was 1 ml/min. Thirty-six fractions were collected for every 30 s and were subsequently dried in a vacuum centrifuge. Elution time of the angiotensin peptides was determined by injections of the synthetic angiotensin peptides into HPLC apparatus recording the absorbance with a spectrophotometer (model 484, Waters) tuned to 214 nm. Typical retention times (in min) were: angiotensin I (15.8), angiotensin II (4.5), angiotensin III (6.8). The amount of angiotensin I and angiotensin II in appropriate fractions were measured by radioimmunoassay.

2.6. Binding assay

The membrane preparation and binding assay were performed based on the method by Edwards et al. (1992). Glomeruli and cortical tubule fractions were prepared from minced renal cortex using sieves of pore sizes of 250, 150, 106, and 75 μm. Tissue remaining on the 150 μm sieve consisted mainly of tubular fragments and tissue remaining on the 75 μm sieve consisted mainly of glomeruli fragments. Protein concentration of prepared membrane was measured by Protein Assay Kit (Nippon Bio-Rad Laboratories, Kanagawa, Japan) using bovine serum albumin standards.

2.7. Drugs and chemicals

Angiotensin peptides and pepstatin were purchased from Peptide Institute, Osaka, Japan. Enalapril maleate and all

other chemicals were purchased from Sigma, St. Louis, MO.

2.8. Statistical analysis

Data are represented as means ± S.E.M. One- or two-way analysis of variance and multiple comparisons were used for statistical analysis. $P < 0.05$ was considered significant.

3. Results

3.1. Protocol A: oral administration of enalapril

On the basis of the water intake, the administered dose of enalapril was calculated to 3.92 ± 0.60 and 4.29 ± 0.29 mg kg^{-1} day^{-1} in WKY and SHR, respectively. There were no statistically significant differences in drug intake between the two strains.

Before the enalapril treatment, there were no differences in mean arterial blood pressure between control and enalapril group either in WKY or in SHR. The oral enalapril treatment for 7 days decreased mean arterial blood pressure by approximately 20 mmHg in SHR, whereas it did not show any change in WKY (Fig. 1). Heart rate was not different between vehicle (308 ± 10 beats/min) and enalapril (319 ± 12 beats/min) groups of SHR. Enalapril treatment did not affect heart rate also in WKY (343 ± 17 beats/min) when compared with vehicle-treated group (310 ± 13 beats/min).

Plasma and renal angiotensin levels are shown in Fig. 2. Renal angiotensin II level was decreased by enalapril treatment in SHR (Fig. 2A). In contrast, the decrease in the renal angiotensin II level was not statistically significant in WKY. Plasma angiotensin II levels were not affected by enalapril in either strain (Fig. 2C). Renal angiotensin I levels were not changed by enalapril treatment in either strain (Fig. 2B). Plasma angiotensin I level showed a marked increase with enalapril in SHR but not in WKY (Fig. 2D).

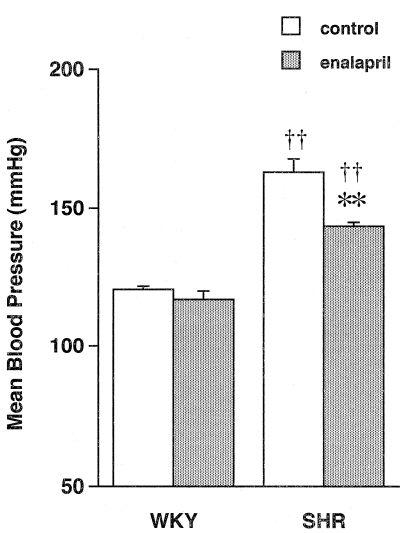

Fig. 1. Bar graph shows the effect of oral enalapril (3 mg kg^{-1} day^{-1}) administration on mean arterial blood pressure in WKY rats and SHR. Each column represents mean ± S.E.M. WKY: control, $n = 8$; enalapril, $n = 5$; SHR: control, $n = 8$; enalapril, $n = 7$. $^{**}P < 0.01$ compared with control rats. $^{††}P < 0.01$ compared with corresponding WKY.

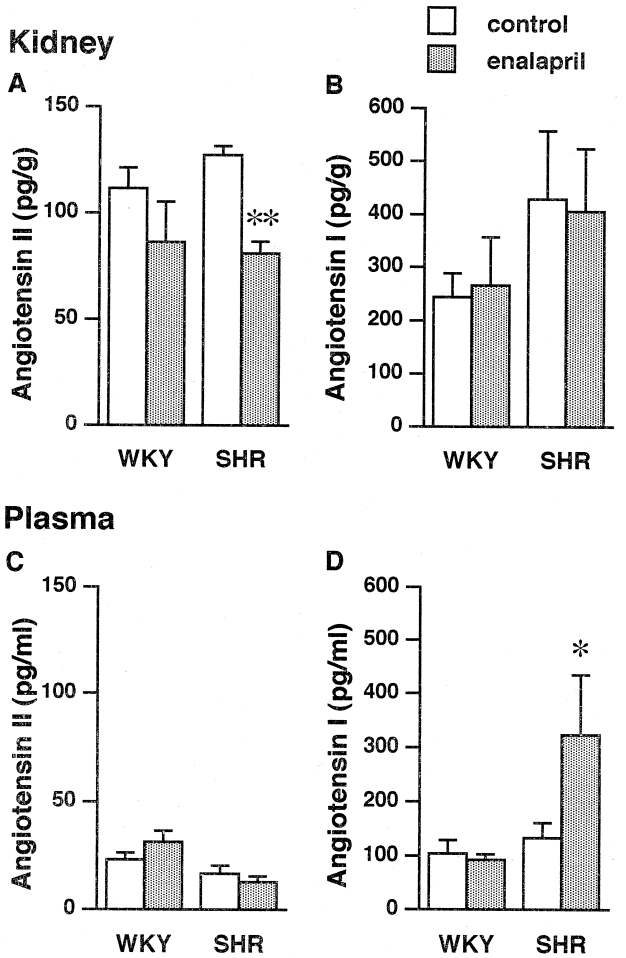

Fig. 2. Upper graphs show the effect of oral enalapril (3 mg kg^{-1} day^{-1}) administration on angiotensin II (A) and angiotensin I (B) concentrations in the kidney of WKY rats and SHR. Lower graphs show angiotensin II (C) and angiotensin I (D) concentrations in plasma of these rats. Each column represents mean ± S.E.M. WKY: control, $n = 8$; enalapril, $n = 5$; SHR: control, $n = 8$; enalapril, $n = 7$. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control rats.

There was no effect of enalapril on the specific binding of [^{125}I]angiotensin II to glomerular membranes either in WKY or SHR. The enalapril treatment decreased B_{max} from 281 ± 20 to 148 ± 14 fmol/mg protein ($P < 0.05$) and K_d value from 1.92 ± 0.22 to 1.23 ± 0.15 nM ($P < 0.05$) in tubules in WKY. In SHR, the enalapril treatment tended to decrease B_{max} in tubules, but showed no statistically significance (295 ± 51 vs. 187 ± 30 fmol/mg protein in control and enalapril groups, respectively). The K_d value in tubular membrane was not affected by enalapril in

SHR. In the preliminary study, we had confirmed that the angiotensin II binding to this membrane preparation was replaced by the angiotensin type 1 (AT_1) receptor antagonist, losartan, but not by angiotensin type 2 receptor antagonist, PD123177.

3.2. Protocol B: renal medullary interstitial infusion of enalapril

Effects of renal medullary interstitial infusion of enalapril on mean arterial blood pressure and heart rate in SHR are shown in Fig. 3. At 3 h of enalapril infusion, there were no differences in mean arterial blood pressure and heart rate in conscious state among vehicle, low dose (0.3 mg kg^{-1} day^{-1}) and high dose (3.0 kg^{-1} day^{-1}) of enalapril-treated groups. Intrarenal infusion of saline induced no significant changes in mean arterial blood pressure (Fig. 3A). Compared to the saline infusion, low dose of enalapril significantly decreased mean arterial blood pressure. High dose of enalapril induced greater decrease

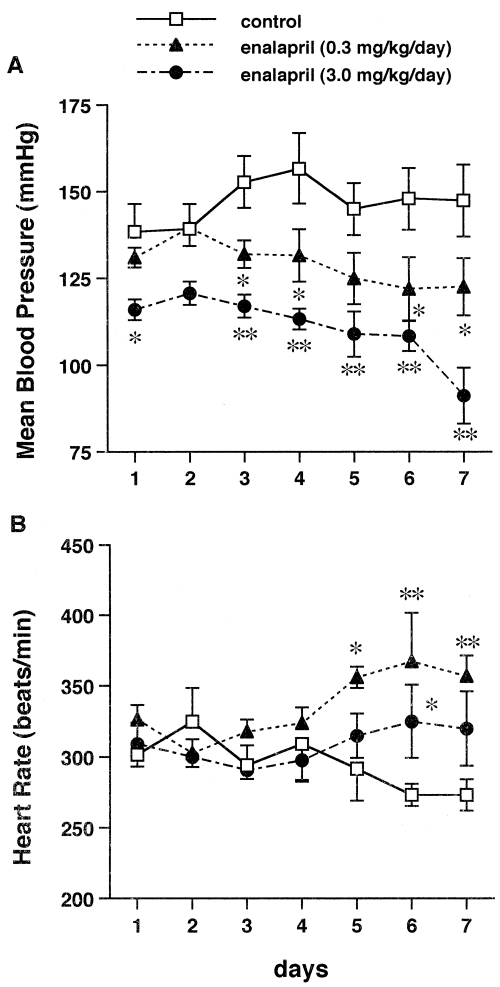

Fig. 3. Line graphs show the effect of intrarenal infusion of vehicle ($n = 6$), low dose (0.3 mg kg^{-1} day^{-1}, $n = 5$) or high dose (3 mg kg^{-1} day^{-1}, $n = 6$) of enalapril on mean arterial blood pressure (A) and heart rate (B) in SHR. Values are means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control rats.

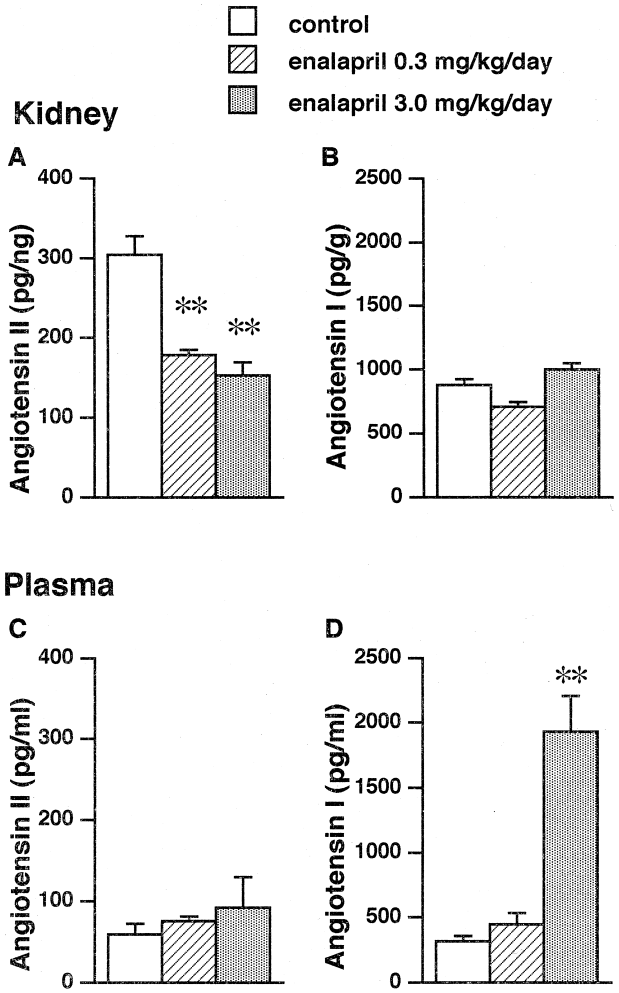

Fig. 4. Upper graphs show the effect of intrarenal infusion of vehicle ($n = 6$), low dose (0.3 mg kg^{-1} day^{-1}, $n = 5$) or high dose (3 mg kg^{-1} day^{-1}, $n = 6$) of enalapril on the angiotensin II (A) and angiotensin I (B) concentrations in the kidney of SHR. Lower graphs show the angiotensin II (A) and angiotensin I (B) concentrations in the plasma of these rats. Each column represents mean ± S.E.M. $^{**}P < 0.01$ compared with control rats.

in mean arterial blood pressure (Fig. 3A). Compared to the saline infusion, low dose of enalapril infusion gradually increased heart rate and the significant differences were observed after the fifth day of the enalapril infusion (Fig. 3B).

The intrarenal infusion of low or high dose of enalapril significantly decreased renal angiotensin II level (Fig. 4A). However, plasma angiotensin II level was not affected by either dose of enalapril (Fig. 4C). Renal angiotensin I levels did not show any changes by these doses of enalapril (Fig. 4B). Plasma angiotensin I level showed a marked increase with high dose of enalapril but not with low dose of enalapril (Fig. 4D).

4. Discussion

In the present study, oral administration of enalapril for 7 days induced hypotension in SHR without affecting

plasma angiotensin II level. The chronic administration of angiotensin converting enzyme inhibitor has been reported to decrease mean arterial blood pressure whereas it did not change (Mento et al., 1989) or rather increased (Mento and Wilkes, 1987) plasma angiotensin II level in rats. Furthermore, in human, the decrease in plasma angiotensin II concentration returns to normal level within 24 h of angiotensin converting enzyme inhibitor treatment although angiotensin converting enzyme inhibition maintains more than 30 h (Juillerat et al., 1990). These and our present results suggest that the chronic hypotensive effect of angiotensin converting enzyme inhibitor cannot be related to the decrease in the circulating angiotensin II level.

One of the candidates that may be responsible for the hypotensive effect of angiotensin converting enzyme inhibitor is the changes in local renin–angiotensin system. The measurement of tissue angiotensin levels could address this hypothesis. In the present study, in contrast to the unchanged circulating angiotensin II level, enalapril treatment decreased tissue angiotensin II level in the kidney accompanied by the hypotension in SHR. Similar dose of enalapril decreased neither blood pressure nor renal angiotensin II level in WKY. The parallel change in blood pressure and angiotensin II level in the kidney was reported by Edling et al. (1995) who compared two angiotensin converting enzyme inhibitors in view of their effects on the components of renin–angiotensin system of several tissues in SHR. When they use the equipotent depressor dose of angiotensin converting enzyme inhibitors, both drugs inhibited angiotensin converting enzyme activity in the kidney to a similar extent, whereas angiotensin converting enzyme inhibition in aorta, heart and lung was significantly greater with moexipril than with enalapril. Campbell et al. (1995) have also reported that oral chronic administration of another angiotensin converting enzyme inhibitor, perindopril, decreased angiotensin II levels in kidney, adrenal, and brown adipose tissue whereas it did not change angiotensin II levels in plasma, aorta, heart, lung, or brain of SHR. These observations are consistent to the hypothesis that the decrease in angiotensin II level in the kidney is important to the hypotensive effect of angiotensin converting enzyme inhibitors. The different effect of angiotensin converting enzyme inhibitors on renal and plasma angiotensin peptides may also indicate the separated regulation of renal and circulating renin–angiotensin system.

To reveal the interaction of renal angiotensin II with hypotensive effect of enalapril, we examined a continuous infusion of enalapril into the renal medulla in SHR. Enalapril infusion (0.3 mg kg^{-1} day^{-1}) decreased mean arterial blood pressure from the third day of the infusion period. At the end of enalapril treatment, the angiotensin II level in the kidney was decreased significantly without changes in plasma angiotensin I and angiotensin II levels. These results may further emphasize the importance of renal angiotensin II level in the hypotensive effect of enalapril. The higher dose of enalapril (3.0 mg kg^{-1} day^{-1}) induced greater decrease in blood pressure and renal angiotensin II level. This dose of enalapril increased plasma angiotensin I level possibly because of the accelerated renin release followed by greater hypotension. The intrarenal infusion was performed according to the method by Lu et al. (1994) which are reported to allow selective infusion of drugs into the renal medulla (Cowley et al., 1995). In our experiment, intrarenal infusion of both doses of enalapril gradually elevated heart rate in accompany with a sustained decrease in mean arterial blood pressure. Angiotensin II is known to affect heart rate through the facilitation of the sympathetic nervous transmission (Vanhoutte et al., 1981; Zimmerman et al., 1984) and inhibition of the vagal activity to the heart (Lumbers et al., 1979). Through the prevention of these effects of angiotensin II, systemic application of enalapril can be thought to prevent tachycardia caused by hypotension. In fact, the heart rate was not affected by oral enalapril treatment in the present study. Therefore, the tachycardia observed in the intrarenal enalapril infusion may suggest that the enalapril was not leak out to the circulation and its effect was concentrated on the kidney. However, during the treatment of higher dose of enalapril, a small amount of the drug may leak out to the systemic circulation and prevent tachycardia because the increase in plasma angiotensin I level was not accompanied by an increase in plasma angiotensin II level.

Cheng et al. (1995) have reported the angiotensin II-induced up-regulation of the renal angiotensin II receptor expression using quantitative polymerase chain reaction method. In captopril-treated rabbits, proximal tubule angiotensin AT_1 receptor mRNA level was decreased whereas glomerular angiotensin AT_1 receptor mRNA level was increased. The incubation of cultured rabbit proximal tubular cell with angiotensin II increased the angiotensin AT_1 receptor mRNA and specific angiotensin II binding (Cheng et al., 1995). On the basis of these results, it was suspected that the renal angiotensin II level may contribute to the angiotensin converting enzyme inhibitor-induced hypotension through the changes in the angiotensin II receptor density. However, in the present study, the changes in the receptor properties were not observed in SHR in spite of the decrease in renal angiotensin II level. In contrast, in the renal cortical tubular membrane of WKY, enalapril decreased angiotensin II receptor density without changes in renal angiotensin II level. A reason for the lack of relationship between angiotensin II level and angiotensin II receptor density may be that the total angiotensin II level in whole kidney measured in our present study does not reflect the local angiotensin II concentration along the tubules. Although the mechanisms for the site and strain different regulation of angiotensin receptor expression is unknown, at least, we consider that the exaggerated hypotensive effect of enalapril in SHR was not attributed to the changes in receptor density or affinity.

In summary, both oral administration and selective intrarenal infusion of enalapril decreased mean arterial blood pressure with concomitant decrease in the renal angiotensin II level without changes in plasma angiotensin II in SHR. The angiotensin II receptor density and affinity in glomerular and tubular membranes of SHR were not affected by enalapril. These results suggest that the circulating and renal renin–angiotensin systems are independently regulated. The suppression of local angiotensin II level in the kidney can be thought to participate in the hypotensive effect of angiotensin converting enzyme inhibitor.

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