Inhibition of Angiotensin Converting Enzyme by CV-3317, a Non-Sulfhydryl Compound

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Abstract—N-[N-{[(S)-1-Ethoxycarbonyl-3-phenylpropyl]-L-alanyl}-N-(indan-2-yl)glycine hydrochloride (CV-3317) and its de-esterified products, CV-3317-COOH and CV-3317-(5-OH)-COOH, inhibited rabbit lung angiotensin converting enzyme (ACE) with the IC50s of 1.2x10^-7, 4.0x10^-8 and 4.9x10^-8 M, respectively, angiotensin I(A-I)-induced vasoconstriction of the rat aorta (IC50: 2.6x10^-7, 2.6x10^-8 and 5.4x10^-8 M, respectively), and A-I-induced pressor response of the rat kidney (IC50: 3.9x10^-7, 3.5x10^-8 and 2.8x10^-8 M, respectively). In these 3 experiments, both de-esterified products were 4 to 14 times more potent than captopril. In rats, CV-3317 (0.0138 to 138 μmol/kg, p.o.) inhibited plasma and lung ACEs, and the effects at a dose of 0.46 μmol/kg lasted more than 8 hr. CV-3317 inhibited the A-I-induced pressor action in rats (0.138 to 13.8 μmol/kg, p.o. or 0.046 to 0.138 μmol/kg, i.v.) and dogs (0.46 to 4.6 μmol/kg, p.o.) in a dose-related manner. CV-3317 was more potent and longer acting than captopril in these in vivo ACE inhibitions. CV-3317 augmented bradykinin-induced hypotension (dogs) and contraction of the ileum (guinea pigs) less potently than captopril. In spontaneously hypertensive rats (SHR), CV-3317 (3 mg/kg, p.o.) markedly inhibited plasma and tissue (aorta, kidney, lung and brain) ACEs; and when administered daily for 2 weeks, it inhibited the plasma, aorta, kidney and lung ACEs; in particular, it markedly inhibited the aortic ACE. Captopril (30 mg/kg, p.o.) markedly inhibited tissue ACEs and slightly plasma ACE, but its inhibitory effects on tissue ACEs, except for the aorta, were unclear by repeated dosings and its effect on plasma ACE was rather enhanced. Thus, the inhibition of vascular ACE may be particularly important for the antihypertensive effect of the ACE inhibitors, including CV-3317, in SHR.

Recent clinical reports indicate that angiotensin converting enzyme inhibitors (CEIs), such as captopril and MK-421 (enalapril), are extremely useful for treating hypertension (1, 2). Although the hypotensive action of CEI is mainly due to the inhibition of the production of the potent vasoconstrictor angiotensin II (A-II) through the inhibition of the angiotensin converting enzyme (ACE), the precise mechanism responsible for reducing blood pressure has not been established. There is not always a correlation between the hypotensive activity of CEIs and the level of plasma renin activity in hypertensive man and animals, and CEIs are effective in hypertensive conditions with normal or suppressed plasma renin activity (3–6). Further, inhibition of the pressor response to angiotensin I (A-I) or inhibition of plasma ACE activity does not always correlate with the blood-pressure-lowering effect produced by CEI. These absences of correlation suggest that the primary site of action of CEI may not be plasma, but tissue ACE. Cohen and Kurz (7) reported that the antihypertensive activity of captopril and
MK-421 in spontaneously hypertensive rats (SHR) correlates better with the inhibition of ACE activity of tissues such as kidney and aorta than with the inhibitory activity of plasma. Miyazaki et al. (8) suggested that vascular ACE may contribute to the regulation of vascular muscle tone and local blood flow by converting A-I into A-II. Captopril is highly effective in treating hypertension, but has serious side effects, which have been attributed to the sulfhydryl moiety of the drug (9). N-[N-{[(S)-1-Ethoxycarbonyl-3-phenylpropyl]-L-alanyl}-N-(indan-2-yl)-glycine hydrochloride (CV-3317) (Fig. 1) is a non-sulfhydryl compound (10) that also lacks the proline moiety present in captopril. CV-3317 is an ethyl ester that is converted in vivo into its active metabolic products, CV-3317-COOH and CV-3317(5-OH)-COOH, and an inactive product, diketopiperazine (DKP)-COOH and so on (11). This report describes the ACE inhibitory activity of CV-3317 and its metabolic products in comparison with captopril. In a previous paper (12), we describe that CV-3317 exerts a potent antihypertensive action in SHR and renal hypertensive rats and dogs.

Materials and Methods

Inhibition of angiotensin converting enzyme (ACE)

Rabbit lung ACE: ACE was prepared from the lung of male albino rabbits according to the slightly modified method (used 100 mM borate HCl-300 mM sodium chloride buffer instead of 500 mM potassium phosphate-300 mM sodium chloride buffer) of Wallace et al. (13). The enzyme activity was determined by spectrophotometric assay of hippuric acid, the hydrolytic product of the
synthetic substrate hippuryl-L-histidyl-L-leucine (HHL), as described by Cushman and Cheung (14). The final assay medium contained 5 mM HHL and the supernatant (20,000xg, 25 min) of lung homogenates in the borate-NaCl buffer (250 µl) in the presence or absence of drugs (0.01 to 10 µM), and it was incubated at 37°C for 60 min. After the reaction was terminated by adding 1N HCl (150 µl), the hippuric acid formed was extracted with 2 volumes of ethyl acetate and determined from its absorbance at 228 nm. The IC50 values (the concentration of drugs required for the 50% inhibition of ACE activity) were calculated.

Rat plasma and tissue ACEs: Male Sprague-Dawley (Jcl) rats, 6 to 7 weeks old, and male SHR (SHR:Ta), 18 to 22 weeks old, were used. At given times following the oral administration of the ACE inhibitor, the rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Blood was withdrawn from the abdominal aorta by a heparinized syringe, and plasma was separated by centrifugation (3,000 rpm, 10 min). Lung tissue was homogenized with 4 volumes of the borate-NaCl buffer using a polytron homogenizer. Plasma and the supernatant (20,000xg, 1 hr) of the lung homogenates were used for the ACE assay described above. In the SHR, the lung, thoracic and abdominal aortae, left kidney, and cerebral cortex were excised, and the tissues were homogenized with 9 volumes of the borate-NaCl buffer using a polytron (lung, kidney and brain) or a teflon homogenizer (aorta). The enzyme activities in plasma and tissues were measured by a radioenzymatic assay, as described by Rohrbach (15). Plasma (20 µl) or tissue homogenates, containing 0.2 mg of the lung and 2 mg of the aorta, kidney, and brain, were incubated with 14C-HHL (500 nmol, 0.05 µCi/tube) in 100 µl final incubation volume at 37°C for 30 to 60 min. After the reaction was stopped by adding 50 µl 1N HCl, the 14C-hippuric acid formed was extracted with 400 µl ethyl acetate, and the radioactivity in the extract was measured with a liquid scintillation spectrometer (LKB 1216 Rackbeta, U.S.A.). The ACE activity was expressed as the production rate of 14C-hippuric acid (nmol/min/ml or mg wet weight). The assay for plasma and tissue ACEs was performed within 3 hr of their preparation, since captopril has been reported to be unstable on storage of tissue samples (7).

Inhibition on angiotensin I (A-I)-induced vasoconstriction and pressor response

Rat aortic ring preparations: The descending thoracic aorta excised from male Sprague-Dawley rats, 9 to 11 weeks old, was cut into ring segments of about 4 mm width, and the preparation was mounted in an organ bath containing 20 ml of Krebs-Henseleit solution, bubbled with 95% O2-5% CO2 gas at 37°C. A resting tension of 10 g was applied. After equilibration for about 1 hr, the tension developed was isometrically recorded on an ink-writing polygraph recorder (R-1500, Nihon Kohden, Japan) via a force displacement transducer (TB-611T, Nihon Kohden, Japan). The concentration-response relation was achieved by cumulatively administering 5×10⁻¹⁰ to 10⁻⁶ M of A-I. The inhibitory effects of CEIs on the contraction produced by a submaximum concentration (5×10⁻⁸ M) of A-I were then examined. CEIs, at concentrations of 10⁻⁸ to 10⁻⁶ M, were added to the incubation medium 10 min before the injection of A-I. The ACE inhibitory activity was expressed as IC50 values: the concentration of CEIs necessary to produce a 50% reduction of the vasoconstrictive response to A-I. In some experiments, the effect of CEIs on the contraction of the ring preparation induced by A-II was examined.

Perfused rat kidney preparations: According to the method of Shibouta et al. (16), the kidneys isolated from male Sprague-Dawley rats, 7 to 11 weeks old, were perfused with the oxygenated Krebs-Henseleit solution (37°C) at flow rates of 2.5 to 3.5 ml/kidney/min (perfusion pressure: 25 to 55 mmHg). A-I (100 ng/0.1 ml/kidney) was injected into the renal artery during the control period. CEIs (10⁻⁸ to 10⁻⁶ M) were then infused at a rate of 0.1 ml/min into the perfusion system close to the kidney over a period of 2 min, and A-I was injected again. The ACE inhibitory activity was expressed as IC50 values: the concentration of CEIs necessary to produce a 50% reduction of the pressor response to A-I.
Augmentation of bradykinin (BK)-induced contraction of guinea-pig ileum

About 1.5 cm segments of the terminal ileum isolated from male Hartley strain guinea-pigs were mounted in an organ bath containing 20 ml of Tyrode's solution saturated with 95% O₂-5% CO₂ gas (37°C). A resting tension of 1 g was applied. After equilibration for about 1 hr, contraction of the segment was monitored with an isotonic transducer (ME-4012, MEC, Japan) connected to a recorder (B-64, Rikadenki, Japan). CEIs (10⁻⁹ to 10⁻⁵ M) were added to the incubation medium 2 min before the BK application. The ACE inhibitory activity was expressed as AC100 values: the concentration of CEIs necessary to produce a 100% augmentation of contraction of the ileum in response to BK.

Inhibition of pressor response to A-I and augmentation of depressor response to bradykinin (BK)

Rats: On the day before the experiment, male Sprague-Dawley rats, 8 to 10 weeks old, were cannulated with a polyethylene tube into the aorta via the femoral artery (PE-10) for blood pressure measurement and into the vena cava via the femoral vein (PE 50) for injection of drugs. The animals were placed into plastic cages and allowed freedom of movement. A-I (300 ng/kg) was injected into the femoral vein twice during the control period. CEIs were then administered orally at doses of 0.138 to 13.8 μmol/kg/5 ml of water as a suspension with a small amount of gum arabic or administered i.v. at doses of 0.046 and 0.138 μmol/kg as a solution with saline. A-I challenges were repeated at set times thereafter. The pressor response to A-I was measured with a recorder (Sanei 8S62ME Japan) using a pressure transducer (Sanei 45277, Japan). The inhibition of the pressor responses to A-I induced by CEIs was calculated.

Dogs: Conscious normotensive beagle dogs of either sex, weighing 10 to 14 kg, were used in groups of 3 to 4 animals each. The dogs were chronically cannulated with a polyethylene tube (PE-100) into the aorta for blood pressure measurement and into the inferior vena cava for injection of drugs. CEIs were administered orally (as a capsule) at doses of 0.46 to 4.6 μmol/kg. A-I (150 ng/kg) or BK (250 ng/kg) was injected i.v. before and after the CEIs were administered. The procedures taken thereafter were similar to those taken with rats.

Data analysis

All results in the text are expressed as the mean±S.E.M. The values among the different groups were analyzed using the one-way analysis of variance and Dunnett’s test. P values less than 0.05 are considered significant.

Drugs

Drugs used in this study were angiotensin I, angiotensin II, bradykinin and hippuryl-L-histidyl-L-leucine (Protein Research Foundation), hippuric acid (Sigma), ¹⁴C-hippuryl-L-histidyl-L-leucine (sp. act. 3.0–3.2 mCi/mmol) (New England Nuclear), heparin (Shimizu Pharmaceutical Co.), EDTA-2Na (Dojin Chemicals), and pentobarbital-Na (Somnopentyl, Pitman Moore). CV-3317, CV-3317-COOH, CV-3317-(5-OH)-COOH, diketopiperazine (DKP)-COOH and captopril were supplied from the Chemistry Laboratories of this Division.

Results

1. In vitro ACE inhibitory activity

1-1. Rabbit lung ACE

Figure 2 and Table 1 show the inhibitory activity of CV-3317 and its 3 metabolites (see Fig. 1), CV-3317-COOH, CV-3317-(5-OH)-COOH and DKP-COOH and captopril on the lung ACE. The agents except for DKP-COOH...
Table 1. Comparison of the inhibitory activity of CV-3317, its metabolites and captopril on angiotensin converting enzyme

|                  | ACE of rabbit lung: IC50 (M) | Potency | Excised guinea pig ileum: BK, AC100 (M) | Potency |
|------------------|-----------------------------|---------|----------------------------------------|---------|
| CV-3317          | $1.2 \times 10^{-7}$        | 4.8     | $3.9 \times 10^{-7}$                   | 0.004   |
| CV-3317-COOH     | $4.0 \times 10^{-6}$        | 14.5    | $3.6 \times 10^{-9}$                   | 0.44    |
| CV-3317-(5-OH)-COOH | $4.9 \times 10^{-6}$    | 11.8    | $5.9 \times 10^{-9}$                   | 0.27    |
| DKP-COOH         | $>1 \times 10^{-4}$         | <0.006  | -                                      | -       |
| Captopril        | $5.8 \times 10^{-7}$        | 1       | $1.6 \times 10^{-9}$                   | 1       |

The numbers of experiments were 2 to 7 for the agents.

inhibited ACE in a concentration-related manner. CV-3317, CV-3317-COOH and CV-3317-(5-OH)-COOH were 4.8, 14.5 and 11.8 times, respectively, more potent than captopril. The diacid of CV-3317 was considerably more potent than the ester, implying that the diacid represents the active form of the drug. DKP-COOH even at a high concentration of $10^{-4}$ M had little effect on the lung ACE.

1-2. Inhibition of A-I-induced vasoconstriction and pressor response

Rat aortic preparations: A-I at concentrations of $5 \times 10^{-10}$ to $10^{-6}$ M induced a dose-related contraction of the aortic ring preparations; a maximum contraction was achieved at $10^{-6}$ M. Figure 3a shows the inhibitory effect of CEIs on the contraction induced by a submaximum concentration of A-I ($5 \times 10^{-8}$ M). The IC50 values were $2.6 \times 10^{-7}$ M for CV-3317, $2.6 \times 10^{-8}$ M for CV-3317-COOH, $5.4 \times 10^{-8}$ M for CV-3317-(5-OH)-COOH and $2.0 \times 10^{-7}$ M for captopril, demonstrating that CV-3317 was slightly less potent than captopril, and CV-3317-COOH and CV-3317-(5-OH)-COOH were 4 to 8 times more potent than captopril. CV-3317-COOH at a concentration of $10^{-7}$ M had no effect on the contraction induced by A-II ($10^{-10}$ to $10^{-7}$ M) (data not shown).

Perfused rat kidney preparations: A-I at a dose of 100 ng/kidney elicited marked increases of 65 mmHg as a mean value in perfusion pressure. As shown in Fig. 3b, the IC50 values were $3.9 \times 10^{-7}$ M for CV-3317, $3.5 \times 10^{-8}$ M for CV-3317-COOH, $2.8 \times 10^{-8}$ M for CV-3317-(5-OH)-COOH and $3.2 \times 10^{-7}$ M for captopril, demonstrating that CV-3317 was slightly less potent than captopril, and CV-3317-COOH and CV-3317-(5-OH)-COOH were 9 to 11 times more potent than captopril.

1-3. Augmentation of BK-induced contraction of ileum preparations

BK ($10^{-9}$ to $10^{-8}$ M) induced a concentration-related contraction of the ileum. Table 1 shows the augmenting effect of CEIs on the contraction induced by BK ($3 \times 10^{-9}$ M). CV-3317-COOH and CV-3317-(5-OH)-COOH were 1/2 and 1/4 times, respectively, as potent as captopril in the augmenting effect. The diacids of CV-3317 were about...
1000 times more potent than the ester.

2. In vivo ACE inhibitory activity
   2-1. Rat plasma and lung ACE

Dose-response curve: Figure 4 shows the dose-response curves of the inhibitory action of CEIs on rat plasma and lung ACEs 1 hr after oral administration. CV-3317 and captopril at doses above 0.0138 and 13.8 μmol/kg, respectively, significantly inhibited plasma ACE in a dose-related manner. The doses inhibiting plasma ACE by 50% (ID50: μmol/kg, p.o.) were 0.0431 for CV-3317 and 10.4 for captopril. CV-3317 and captopril at doses above 0.138 and 138 μmol/kg, respectively, significantly inhibited lung ACE; the ID50 values were 0.158 for CV-3317 and 153 μmol/kg for captopril.

Time course of inhibition: Figure 5 shows time course of the inhibitory action of orally administered CV-3317 (0.46 μmol/kg) and captopril (138 μmol/kg) on plasma and lung ACEs. Each drug maximally inhibited both ACE activities 1 hr after the administration (Fig. 4). The duration of inhibitory action was expressed as the time (T1/2) that the inhibited ACE recovers to half the pre-administration level of ACE; these were 7.5 hr for CV-3317 and 5.5 hr for captopril in plasma ACE and 16 hr for CV-3317 and 6 hr for captopril in lung ACE, demonstrating that CV-3317 is longer acting than captopril.

2-2. Inhibition of pressor response to A-I and augmentation of depressor response to BK

Rats: As shown in Fig. 6a, CV-3317 and captopril at oral doses of 0.138 to 13.8 μmol/kg inhibited the pressor response to A-I dose-dependently. The agents had no effect on the pressor response to A-II (data not shown). CV-3317 at a dose of 13.8 μmol/kg was more potent than captopril. The ID50 values calculated from the area under the curve for 0 to 4 hr were 1.9 and 5.4 μmol/kg for CV-3317 and captopril, respectively. CV-3317-COOH at a dose of 13.8 μmol/kg had little effect on the A-I-induced pressor action, suggesting a poor enteral absorption of the de-esterified form. When CEIs were administered i.v. (Fig. 6b), each drug maximally inhibited the pressor response to

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**Fig. 4.** Dose-response curves of the inhibitory action of CV-3317 and captopril on rat plasma and lung angiotensin converting enzyme (ACE). The plasma and lung ACE activity was measured 1 hr after the oral administration of the agents. ■: the mean±S.E.M. for 4–6 rats treated with vehicle. Each point consists of 3–8 experiments. ●, CV-3317; □, Captopril. The 138 μmol of captopril corresponds to 30 mg. Dunnett's test: vs. vehicle, *P<0.05, **P<0.01.

**Fig. 5.** Time course of the inhibitory action of CV-3317 and captopril on rat plasma and lung angiotensin converting enzyme (ACE). HA: hippuric acid. ●, CV-3317 (0.46 μmol/kg) (n=3–6); □, Captopril (138 μmol/kg) (n=3–8). Dunnett's test: vs. vehicle, *P<0.05, **P<0.01.
Fig. 6. Inhibitory action of CV-3317, its metabolites and captopril on angiotensin-I(A-I)-induced pressor action in the rat. A-I: 300 ng/kg, i.v. Each point consists of 2–12 experiments. •, CV-3317; ○, CV-3317-COOH; ▲, CV-3317-(5-OH)-COOH; ■, Captopril.

Fig. 7. Inhibitory action of CV-3317 and captopril on angiotensin-I(A-I)-induced pressor action and augmenting action on bradykinin(BK)-induced depressor action in the dog. A-I: 150 ng/kg, i.v. BK: 250 ng/kg, i.v. Each point consists of 3–5 experiments. •, CV-3317; ■, Captopril.

A-I 5 min after the injection, and then the effect rapidly diminished. The active metabolic products, CV-3317-COOH and CV-3317-(5-OH)-COOH, at a dose of 0.138 μmol/kg showed a similar activity to CV-3317. Captopril (0.138 μmol/kg) was much less potent than these CV-compounds.

Dogs: The inhibitory effect of CV-3317 on the pressor response to A-I was more potent in both efficacy and duration than that of captopril (Fig. 7a). In contrast, CV-3317 augmented the depressor effects of BK less potently than captopril (Fig. 7b). The p.o. doses producing a 50% reduction of the pressor response to A-I (ID50) were 1.1 μmol/kg for CV-3317 and 1.8 μmol/kg for
Fig. 8. Antihypertensive action and plasma and tissue angiotensin converting enzyme (ACE) inhibitory action of CV-3317 and captopril in spontaneously hypertensive rats (SHR). Blood pressure was measured in 7-14 rats for each group and the ACE activity was measured in 3-6 rats for each point. Bar: the mean ±S.E.M. for 25-26 rats treated with vehicle. ○, CV-3317 (3 mg/kg, p.o.); □, Captopril (30 mg/kg, p.o.); ○, vehicle. Dunnett's test: vs. vehicle, *P<0.05, **P<0.01.

captopril, whereas the dose augmenting the depressor response to BK by 2 times (AD100) was 2.8 μmol/kg for CV-3317 and 0.46 μmol/kg for captopril.

3. Antihypertensive action and plasma and tissue ACE inhibitory action in SHR

As shown in Fig. 8, when CV-3317 (3 mg/kg/day) and captopril (30 mg/kg/day) were administered orally to SHR for 2 weeks and blood pressure was measured by the tail cuff method, the antihypertensive action of CV-3317 was increased in potency and duration and that of captopril was not; both agents at these doses were equipotent at the point of 2 and 5 hr on the 14th day. In a single administration, CV-3317 markedly inhibited the ACE activity of plasma and all tissues (the aorta, kidney, lung and brain), whereas captopril markedly inhibited the tissue ACEs and slightly inhibited plasma ACE. When the 2-week-administration was completed, CV-3317 continued to inhibit the aortic, kidney and lung ACEs: the inhibitory effect on the aortic ACE was especially marked, and captopril markedly inhibited only the aortic ACE. The plasma ACE activity was enhanced by the consecutive administration of captopril.

Discussion

The present in vitro and in vivo experiments demonstrate that CV-3317 and its diacid products, CV-3317-COOH and CV-3317-(5-OH)-COOH, are potent ACE inhibitors. The diacid inhibited rabbit lung ACE (IC50; 4.0 to 4.9×10^-8 M) more potently than CV-3317 (IC50: 1.2×10^-7 M), indicating that CV-3317 is a prodrug which is hydrolyzed in vivo into its active diacid form (11). The inhibitory activity of captopril (5.8×10^-7 M) on rabbit lung ACE is compatible with the results reported by Gross et al. (17).

In rabbit lung preparations, the activity of ACE, which is a Zn++ requiring enzyme, was inhibited with EDTA and reversed by adding
Zn++. In contrast, the inhibition of ACE with CV-3317-COOH or captopril was not reversed with Zn++, indicating that the action of these agents is not due to their chelating action on Zn++. (data not shown). The inhibitory effect on rat plasma ACE caused by the oral administration of CV-3317 was not changed by keeping the plasma samples at -20°C for 3 weeks, whereas the effect caused by captopril was markedly reduced by storage of the plasma samples at -20°C (data not shown). Therefore, the assay for ACEs of all samples from captopril-treated rats was performed within 3 hr of their preparation, as described by Cohen and Kurz (7).

The experiments on ACE inhibitory activity estimated by inhibition of the A-I induced vasoconstriction in the aortic ring or by inhibition of the A-I induced pressor action in the perfused kidney demonstrate that the diacid of CV-3317 is 4 to 11 times more potent than captopril (Fig. 3, a and b). CV-3317-COOH, when administered orally, was much less potent than the parent CV-3317, indicating a poor enteral absorption of the de-esterified form.

ACE catalyzes both the conversion of A-I to A-II and the inactivation of BK (18). The active metabolites of CV-3317 augmented the BK-induced contraction of the guinea-pig ileum less potently than captopril (Table 1), whereas they inhibited the ACE activity in other in vitro and in vivo systems more potently than captopril (Table 1 and Figs. 2 to 6). CV-3317 itself also inhibited more sensitively the conversion of A-I to A-II than the inactivation of BK. These findings could explain why CV-3317 was less potent in augmenting BK-induced hypertension in vivo than captopril in dogs (Fig. 7). The reasons for this dissociation of A-I conversion and BK-inactivation between CV-3317 and captopril may be due to the difference in the kinetic characteristics of ACE depending on the substrates and the difference in multiple binding properties of inhibitors to ACE (19).

In SHR, the daily administration of CV-3317 for 2 weeks increased its inhibitory activity on the aortic ACE, but not on the plasma, kidney, lung or brain ACE and enhanced its antihypertensive activity. In contrast, the consecutive treatment with captopril enhanced the plasma ACE activity and diminished its inhibitory effect on other tissue ACEs, except for the aortic ACE, the activity of which remained unchanged without affecting its antihypertensive action. These findings suggest that the inhibition on the aortic ACE may be important for the continual blood pressure lowering effect of ACE inhibitors, as Unger et al. (20) suggested.

It has been reported that in SHR, the level of plasma renin is normal and the levels of vascular renin and ACE are high (21, 22). In 2-kidney, 1 clip hypertensive rats, the level of plasma renin, vascular renin and vascular ACE are high (23); and in 1-kidney, 1 clip hypertensive rats, the level of vascular renin is high (24), but vascular ACE is normal (25). CV-3317 and captopril elicited an antihypertensive action only in SHR and 2-kidney, 1 clip hypertensive rats with a high vascular ACE activity (12). These findings suggest that the antihypertensive effect of CV-3317 and captopril is closely related to the inhibition of vascular ACE.

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