Antihypertensive Action of a New Angiotensin Converting Enzyme Inhibitor, (R)-3-[(S)-1-Carboxy-5-(4-Piperidyl)pentyllamino-4-Oxo-2,3,4,5-Tetrahydro-1,5-Benzothiazepine-5-Acetic Acid (CV-5975), in Various Hypertensive Models

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Abstract—The antihypertensive activity of a new angiotensin converting enzyme (ACE) inhibitor, CV-5975, (R)-3-[(S)-1-carboxy-5-(4-piperidyl)pentyllamino-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-5-acetic acid, was examined in normotensive rats and various hypertensive animal models. In spontaneously hypertensive rats, CV-5975 (1 to 10 mg/kg, p.o.) had a dose-related, sustained antihypertensive action, which was more potent and longer than that of enalapril. The potency and duration of action of CV-5975 was intensified when it was administered repeatedly or combined with hydrochlorothiazide. CV-5975 (1 mg/kg, p.o.) inhibited the ACE activity of plasma and tissues; inhibition on the ACE activity of the aorta, kidney, and brain was marked when CV-5975 was administered repeatedly. In 2-kidney, 1 clip hypertensive rats (1 to 10 mg/kg, p.o.) and dogs (0.3 and 1 mg/kg, p.o.), CV-5975 had a marked, sustained antihypertensive action, which was more marked than that of enalapril. In normotensive rats (10 mg/kg), 1-kidney, 1 clip hypertensive rats (3 and 10 mg/kg), and hyporeninemic DOCA/salt hypertensive rats (1 to 10 mg/kg/day), CV-5975 administered orally once or repeatedly reduced blood pressure, whereas enalapril did not. These results indicate that CV-5975 is a potent and long-lasting antihypertensive agent, the action of which is mediated primarily by inhibiting ACE activity and partly by some unknown mechanisms.

The renin-angiotensin system plays an important role in the homeostatic mechanisms that regulate both arterial blood pressure and salt and water balance, and it is involved in the pathogenesis of several forms of experimental and human hypertension (1, 2). The pharmacologic modification of the renin-angiotensin system by inhibiting angiotensin converting enzyme (ACE) activity provides an important approach for treating certain cardiovascular diseases. Long term trials to control hypertensive diseases through regulating this system have led to the discovery of orally active ACE inhibitors, exemplified by captopril (3–5). Recent clinical research indicates that this class of agent is extremely useful for treating hypertension (6, 7) and may be suitable for treating congestive heart failure (8). (R)-3-[(S)-1-carboxy-5-(4-piperidyl)pentyl]amino-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepine-5-acetic acid (CV-5975) is a new non-sulfhydryl ACE inhibitor. The synthetic design and ACE inhibitory action of CV-5975 have been described in preceding papers (9, 10). In this report, we describe the antihypertensive activity of CV-5975 compared with that of enalapril in normotensive rats and various hypertensive animal models.

Materials and Methods

1. Experimental animals

1.1. Rats
Normotensive rats: Male Wistar Kyoto rats (WKY:Ta), 20 to 22 weeks old, were used.

Spontaneously hypertensive rats: Male spontaneously hypertensive rats (SHR:Ta), 20 to 22 weeks old, were used.

Renal hypertensive rats: Six week old male Wistar rats (Wistar:Jcl) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). A dorsal incision was made, and the left renal artery was constricted by applying a silver clip (internal diameter, 0.22 to 0.27 mm). The right kidney was left intact for 2-kidney, 1 clip hypertensive rats (2K, 1C-RHR) and removed for 1-kidney, 1 clip hypertensive rats (1K, 1C-RHR). After surgery, the rats were maintained on a standard diet (CE-2, Japan Clea Laboratories) and tap water ad libitum for 4 to 6 weeks. Rats with systolic blood pressure of 160 to 240 mmHg (measured by the tail cuff method) were used.

DOCA/salt hypertensive rats: Six week old male Wistar rats (Wistar:Jcl) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The left kidney was removed, and a 25 mg pellet of desoxycorticosterone acetate (DOCA) was immediately implanted into the dorsum of the rat. The rats were maintained on a standard diet (CE-2), tap water, and 1% NaCl solution ad libitum for 4 to 6 weeks. Rats with systolic blood pressure of 160 to 240 mmHg (measured by the tail cuff method) were used.

1.2. Dogs

Renal hypertensive dogs: Male beagle dogs (13 to 16 kg) were used. Under sodium pentobarbital (30 mg/kg, i.v.) anesthesia, the left renal artery was constricted with a silver clip to reduce renal blood flow by about 80% for 2-kidney, 1 clip hypertensive dogs (2K, 1C-RHD). The dogs were used 2 to 5 weeks after surgery.

2. Experimental protocol

2.1. Single administration

Rats: Rats (normotensive rats, SHR, 2K, 1C-RHR, 1K, 1C-RHR and DOCA/salt hypertensive rats) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and the abdominal aorta was cannulated with a polyethylene tube (PE-10 fused to PE-50) via the femoral artery. A catheter was passed subcutaneously and exteriorized on the neck; this was filled with saline containing heparin. The catheter was kept in place by a harness and spring and attached to water tight swivels (Instech 375/22, U.S.A.). The animals were allowed to recover for 1 to 2 days in individual plastic cages. The catheter was connected to a pressure transducer (Sanei, 45277, Japan), and blood pressure was recorded with a pen-writing oscillograph (Sanei, 8562E, Japan) for 24 hr after the drugs were administered. The animals were allowed free access to a standard diet (CE-2) and tap water during the experiments. CV-5975 (1 to 10 mg/kg) or enalapril (10 mg/kg) was administered orally in a volume of 2 ml/kg as a solution in water or as a suspension with gum arabic. Control rats were given vehicle (2 ml/kg of water) only.

Dogs: Systolic and diastolic blood pressure were measured indirectly with a cuff and transducer placed on the antebraclial artery, and heart rate was calculated from blood pressure pulse. The systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) before the drug was administered were about 200 mmHg, 100 mmHg, and 110 beats/min, respectively. Test compounds were given orally at 0.3 and 1 mg/kg as a solution in water (10 ml/kg) and the changes in blood pressure and heart rate were monitored for 24 hr.

2.2. Repeated administration

SHR and DOCA/salt hypertensive rats were used in these experiments. The rats were given the test compounds at the dose of 1, 3 or 10 mg/kg/2 ml of water orally once a day at between 9:00 and 10:00 a.m. for 2 weeks. Blood pressure was measured by the tail cuff method (37°C, 6 to 9 min) before; at 5, 10 and 24 hr after the 1st, 3rd, 7th and 14th doses; and several times after drug administration was stopped; heart rate was measured with a pulse rate meter.

2.3. Combination with hydrochlorothiazide

CV-5975 (1 mg/kg, p.o.) and hydrochlorothiazide (10 mg/kg, p.o.) were administered concurrently to 20 week-old SHR every day for 7 days. Blood pressure was measured by the tail cuff method (37°C, 6 to 9 min) before; at 5, 10 and 24 hr after the 1st, 3rd, 7th and 14th doses; and several times after drug administration was stopped; heart rate was measured with a pulse rate meter.
2.4. Inhibition of plasma and tissue ACE activity in SHR

At given times after CV-5975 or enalapril was administered orally to male SHR (SHR: Ta), they 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). The lung, thoracic, and abdominal aorta, left kidney and cerebral cortex were removed, and the tissues were homogenized with 9 volumes of borate-NaCl buffer using a polytron (lung, kidney and brain) or a teflon homogenizer (aorta). The enzyme activities in plasma and tissues were measured as described below.

3. Assay methods

ACE: The enzyme activity in plasma and tissues was measured by a radioenzymatic assay, as described by Rohrbach (11). 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-hippuryl-L-histidyl-L-leucine (14C-HHL, 500 nmol, 0.05 μCi/tube) in a 100 μl incubation volume at 37°C for 30 to 60 min. After the reaction was stopped by adding 50 μl 1 N HCl and the 14C-hippuric acid that formed was extracted with 400 μl ethyl acetate, the radioactivity in the extract was measured using 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 of plasma or mg protein).

Renin: The concentration of plasma renin (PRC) and vascular renin (VRC) was determined by a radioimmunoassay of angiotensin I (A-I). Plasma or aorta homogenates were mixed with angiotensinogen, 8-hydroxyquinoline, 2,3-dimercaptopropanol and phenylmethylsulfonyl fluoride, and the mixture was incubated at 37°C. The angiotensin I generated was measured with a radioimmunoassay kit (CEA-IRA-SORIN, France). Renin concentration was expressed as ng of angiotensin I generated in 1 hr by 1 ml of plasma or aorta tissue equivalent to 1 mg protein. Angiotensinogen was prepared from the plasma of nephrectomized rats by the method of Haas et al. (12).

4. Data analysis

All results are expressed as the mean±S.E.M. Values for the different groups were compared using one- or two-way analysis of variance and Dunnett's test. P values less than 0.05 were considered significant.

5. Drugs

Hydrochlorothiazide (Esidrex powder) was obtained from Ciba-Geigy; desoxycorticosterone acetate from Tokyo-Kasei; heparin from Shimizu Pharmaceutical Co.; EDTA-2Na from Dojin Chemicals; pentobarbital-Na (Somnopentyl) from Pitman Moore; 14C-hippuryl-L-histidyl-L-leucine (sp. act. 3.2 mCi/mmol) from New England Nuclear Co.; and 8-hydroxyquinoline, 2,3-dimercaptopropanol, and phenylmethylsulfonyl fluoride from Sigma. CV-5975 and enalapril were supplied by the Chemistry Laboratories of this Division.

Results

1. Antihypertensive action

1.1. Hypotensive action in normotensive rats

As shown in Table 1, a single oral dose of CV-5975 (10 mg/kg) slightly, but significantly, reduced blood pressure in normotensive rats. The maximum hypotensive effect was maintained for 5 to 10 hr, and blood pressure returned to pre-drug levels 24 hr after

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0 (mmHg)</th>
<th>1</th>
<th>3</th>
<th>5 (mmHg)</th>
<th>7</th>
<th>10</th>
<th>24 hr</th>
</tr>
</thead>
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<tr>
<td>Control</td>
<td>(5)</td>
<td>122±3</td>
<td>0±1</td>
<td>2±1</td>
<td>2±1</td>
<td>1±1</td>
<td>0±1</td>
<td>2±2</td>
</tr>
<tr>
<td>CV-5975</td>
<td>(5)</td>
<td>123±1</td>
<td>−5±1</td>
<td>−4±2</td>
<td>−9±2*</td>
<td>−8±2*</td>
<td>−10±1*</td>
<td>−5±1</td>
</tr>
<tr>
<td>Enalapril</td>
<td>(5)</td>
<td>123±1</td>
<td>0±0</td>
<td>0±1</td>
<td>2±1</td>
<td>2±1</td>
<td>1±1</td>
<td>0±0</td>
</tr>
</tbody>
</table>

Values represent the mean±S.E.M. The dose of both agents: 10 mg/kg, p.o. *: P<0.05, significantly different from the control group.
administration. The same dose of enalapril did not lower blood pressure.

1.2. Antihypertensive action in SHR

Single administration: CV-5975 administered to SHR at 1, 3 and 10 mg/kg, p.o., lowered blood pressure dose-dependently by 10 to 35 mmHg (Fig. 1, 1st dose). The maximum effects were seen 5 to 10 hr after administration, and they lasted for more than 24 hr. The same dose of enalapril had less potent antihypertensive action, (Figs. 1 and 6). The blood pressure returned to pre-drug values within 24 hr of each administration of enalapril.

Repeated administration: CV-5975 and enalapril were administered to SHR for 2 weeks; the blood pressure on the 1st, 3rd, 7th and 14th days are shown in Fig. 1. CV-5975 at 1, 3 and 10 mg/kg, p.o., had a dose-related antihypertensive action, which was intensified in potency and duration by repeated administration; the maximum effect was observed on the 7th day. Enalapril had a dose-related antihypertensive action, which was less potent than that of the corresponding doses of CV-5975. One week after the administration of ACE inhibitors was stopped, the blood pressure in enalapril-treated rats was 206±3, 207±2 and 213±4 mmHg at 1, 3 and 10 mg/kg/day, respectively; these values were similar to the respective control levels (212±3, 213±3 and 216±2 mmHg). In contrast, the blood pressure in CV-5975-treated rats was 202±2, 203±4 and 202±3 mmHg at 1, 3 and 10 mg/kg/day, respectively, demonstrating that the values were kept significantly lower.
than that of the respective control at the time. The blood pressure in CV-5975 treated rats recovered to the control levels at 2 weeks after the administration was stopped.

**Effect of co-administration with hydrochlorothiazide:** As shown in Fig. 2, the repeated administration of hydrochlorothiazide (10 mg/kg) alone to SHR had no effect on blood pressure, and CV-5975 given at 1 mg/kg for 1 week slightly, but significantly reduced blood pressure at each measuring point compared with the untreated controls. When CV-5975 was given daily for 1 week combined with hydrochlorothiazide, the potency and duration of the antihypertensive effect of CV-5975 were intensified; the effects lasted for more than 24 hr. After the treatment was stopped, the blood pressure of rats given CV-5975 alone returned to the control level within 1 week, whereas in rats given the combined treatment, the blood pressure remained lower than that of the controls for up to 2 weeks.

### 1.3. Antihypertensive action in experimental hypertension

#### 1.3.1. 2-Kidney, 1 clip hypertensive rats

The effects of single oral doses of CV-5975 and enalapril (1, 3 and 10 mg/kg, p.o.) on blood pressure are shown in Fig. 3a. CV-5975 caused a dose-related, marked and sustained decrease in blood pressure. The maximum effect (about 65 mmHg) was seen at 7 to 10 hr after dosing, and the return of blood pressure to pre-drug levels required more than 24 hr. The effect of CV-5975 was more potent and longer than that of enalapril (10 mg/kg) (see Fig. 7).

#### 1.3.2. 1-Kidney, 1 clip hypertensive rats

After a single dose of CV-5975 was given at 1, 3 or 10 mg/kg to 1K, 1C-RHR, blood pressure decreased dose-dependently (Fig. 3b). At 10 mg/kg, the effect was seen from 3 hr and blood pressure remained lower than the control level for more than 10 hr. In contrast, enalapril (10 mg/kg, p.o.) had no antihypertensive action (see Fig. 7).

#### 1.3.3. DOCA/salt hypertensive rats

**Single administration:** As shown in Fig. 3c, CV-5975 at 1, 3 or 10 mg/kg induced a mild and consistent antihypertensive effect. In contrast, enalapril at 10 mg/kg had no effect on blood pressure (see Fig. 7).

**Repeated administration:** The blood pressure on days 1, 3, 7 and 14 of the administration of CV-5975 and enalapril are shown in Fig. 4. CV-5975 at 1, 3 or 10 mg/kg reduced arterial blood pressure in a dose-related manner. However, the antihypertensive activity was not altered by repeated administration of CV-5975, and the antihypertensive effect disappeared within 24 hr after each administration of CV-5975 was stopped. Treatment with enalapril at 10 mg/kg did not decrease the blood pressure.

### 1.4. Renal hypertensive dogs

In 2K, 1C-RHD, CV-5975 at 0.3 and 1 mg/kg induced a modest but long-lasting reduction in systemic blood pressure within 1 hr of the administration. The blood pressure remained lower than control levels for more

![Fig. 2. Effect of repeatedly administering CV-5975, or hydrochlorothiazide (HC) or both on systolic blood pressure in spontaneously hypertensive rats. Each point represents the mean±S.E.M. (○). Control (n=7); (■), CV-5975, 1 mg/kg/day (n=7); (△), HC, 10 mg/kg/day (n=7); (♦), CV-5975, 1 mg/kg/day+HC, 10 mg/kg/day (n=7). *: P<0.05, **: P<0.01 significantly different from the control group. +: P<0.05, ++: P<0.01, significantly different from the group receiving CV-5975 alone.](image-url)
than 10 hr. Enalapril given in the same doses was less potent than CV-5975. CV-5975 lowered both systolic and diastolic blood pressure by about 40 mmHg. When CV-5975 was given at 1 mg/kg, p.o., heart rate tended to increase, but the change was not statistically significant. Enalapril at either dose and CV-5975 at a dose of 0.3 mg/kg, p.o., did not affect heart rate (Fig. 5).

2. Plasma and tissue ACE inhibiting action in SHR

   As shown in Fig. 6, when CV-5975 (1 mg/kg/day) was administered to SHR for one week and blood pressure was measured by the tail cuff method (the data on blood pressure in Fig. 6 are from the results in Fig. 1), its antihypertensive action was intensified in potency and duration by repeated administration. CV-5975 administered once markedly inhibited the ACE activity of plasma, kidney, and lung, moderately inhibited activity of the aorta and slightly inhibited activity of the brain. When the one week-administration was completed, CV-5975 markedly and continuously inhibited the ACE activity of the aorta and brain as well as that of plasma, kidney and lung. Enalapril (1 mg/kg) reduced blood pressure moderately only at 5 hr after the 1st and 7th administration, when the ACE activity of the plasma, aorta, lung and kidney, but not the brain, was moderately inhibited.

3. Plasma and vascular renin concentration and ACE activity in various hypertensive rats

   Table 2 summarizes the plasma and vascular renin concentration and ACE activity in various hypertensive animal models. Blood pressure was significantly elevated in SHR at 20 weeks of age, in 1K, 1C-RHR and 2K, 1C-RHR at 4 to 6 weeks after the clips were applied; and in DOCA/salt hypertensive rats at 4 to 6 weeks after surgery. Vascular ACE activities were higher in these hypertensive rats than in the normotensive controls. However, plasma ACE activity was normal except in SHR, which had lower ACE activity than the controls. In SHR, the renin concentration in aortae was about 2.3 times higher than that in normotensive WKY. The renin concentrations of plasma and aorta were also significantly higher in 2K, 1C-RHR than in normotensive rats. In 1K, 1C-RHR, the concentration of renin in aortae, but not in plasma, was higher than that in normotensive rats. The aortic concentration of renin was higher in the aortae of 2K, 1C-RHR than in those of 1K, 1C-RHR. The concentrations of renin in the plasma and aortae of DOCA/salt hypertensive rats were lower than those in normotensive controls, but were similar to those in normotensive controls treated with 1% NaCl for 4 to 6 weeks.

Discussion

CV-5975 is a potent and long-lasting ACE inhibitor, and its inhibitory activity against
the conversion of A-I to angiotensin II (A-II) in vivo is considered to be more potent and longer lasting than that of enalapril. In the present study, the antihypertensive activity
of CV-5975 was examined in normotensive rats and various hypertensive animal models. In normotensive rats, CV-5975, but not enalapril, slightly but significantly reduced blood pressure (Table 1). We reported that high doses of captopril and delapril induce hypotensive action in normotensive rats (13). Investigations with human volunteers and normotensive animals having a normal sodium intake, show that captopril (14), enalapril (15) and ramipril (16) reduce blood pressure. These results indicate that the hypotensive activity of CV-5975 is not qualitatively different from that captopril or delapril under normotensive conditions, but CV-5975 seems to be effective at lower doses than the other ACE inhibitors. The results also indicate that the renin-angiotensin system plays an important role in maintaining blood pressure in normotensive animals and in human subjects with a normal sodium intake.

CV-5975 and enalapril exerted a marked, more sustained antihypertensive action in the hyperreninemic model of hypertension (2K, 1C-RHR) than in other hypertensive animal models (Fig. 7). The reduction in blood pressure by CV-5975 begins rapidly, and it correlates with the onset of inhibition of pressor responses to A-I. An increase in plasma renin levels is considered to be an important contributory factor in developing and maintaining hypertension in this model (17, 18). The antihypertensive effects of CV-5975 in the renin angiotensin dependent model of hypertension may result from a decrease in circulating A-II levels caused by inhibition of ACE activity, and may depend on the pre-administration activity of the renin angiotensin system and the rate of formation of A-II.

A number of investigators have demonstrated that ACE inhibitors reduce blood

![Fig. 6. Antihypertensive action and plasma and tissue angiotensin converting enzyme (ACE) inhibitory action of CV-5975 and enalapril in spontaneously hypertensive rats (SHR). The data on blood pressure was from the results in Fig. 1. (●), CV-5975, 1 mg/kg, p.o.: (▲). Enalapril, 1 mg/kg, p.o. *: P<0.05, **: P<0.01, significantly different from the control group.](image)

### Table 2. Blood and vascular renin angiotensin system in various rat hypertensive models

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>BP (mmHg)</th>
<th>HR (beats/min)</th>
<th>renin (A-I ng/ml/hr)</th>
<th>Plasma ACE (HA nmol/ml/min)</th>
<th>renin (A-I ng/mg pr/hr)</th>
<th>Aorta ACE (HA nmol/mg pr/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTR</td>
<td>287±5</td>
<td>128±3</td>
<td>367±12</td>
<td>19.7±0.6</td>
<td>128±4</td>
<td>363±24</td>
<td>8.3±1.4</td>
</tr>
<tr>
<td>2K, 1C-RHR</td>
<td>236±13</td>
<td>220±13</td>
<td>394±15</td>
<td>83.9±9.1</td>
<td>123±8</td>
<td>930±59</td>
<td>24.7±5.1</td>
</tr>
<tr>
<td>1K, 1C-RHR</td>
<td>264±12</td>
<td>206±8</td>
<td>400±13</td>
<td>20.7±1.5</td>
<td>136±4</td>
<td>450±38</td>
<td>29.3±1.7</td>
</tr>
<tr>
<td>NTR+NaCl</td>
<td>306±7</td>
<td>126±3</td>
<td>367±10</td>
<td>2.5±0.6</td>
<td>131±4</td>
<td>208±9</td>
<td>4.5±1.1</td>
</tr>
<tr>
<td>DOCA/salt</td>
<td>283±5</td>
<td>217±7</td>
<td>360±8</td>
<td>3.4±0.4</td>
<td>105±6</td>
<td>210±16</td>
<td>26.8±3.1</td>
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<tr>
<td>WKY</td>
<td>402±5</td>
<td>129±2</td>
<td>378±6</td>
<td>21.0±0.6</td>
<td>124±2</td>
<td>317±11</td>
<td>18.9±1.4</td>
</tr>
<tr>
<td>SHR</td>
<td>291±8</td>
<td>197±5</td>
<td>378±18</td>
<td>15.8±0.9</td>
<td>67±6</td>
<td>717±32</td>
<td>41.5±2.9</td>
</tr>
</tbody>
</table>

Values represent the mean±S.E.M. of the results for 7 rats. A-I: angiotensin I; HA: hippuric acid; pr: protein; NTR: normotensive rats; 2K, 1C-RHR: 2 kidney, 1 clip hypertensive rats; 1K, 1C-RHR: 1 kidney, 1 clip hypertensive rats; NTR+NaCl: 1% NaCl treated normotensive rats; DOCA/salt: DOCA/salt hypertensive rats; WKY: Wistar Kyoto rats; SHR: spontaneously hypertensive rats.
pressure in SHR (5, 16, 19-22). In this study, CV-5975 and enalapril in SHR produced a marked, sustained reduction in blood pressure, in which the plasma renin concentrations and ACE activity were not elevated (Fig. 1 and Table 2). These data suggest that the inhibitors may reduce blood pressure in a manner independent of the inhibition of plasma ACE activity in SHR. Alternatively, the antihypertensive effect of the ACE inhibitor may result from the inhibition of ACE activity at other sites. The arterial wall renin concentration and ACE activity were significantly higher in SHR than in normotensive WKY (Table 2). In SHR, the antihypertensive action of CV-5975 was intensified in potency and duration by repeated administration (Fig. 1). A prolonged inhibition of ACE activity was observed in the vascular tissue, exemplified by the aorta, after repeated treatment with CV-5975 (Fig. 6). It has been reported that the inhibitory action on vascular ACE activity is prolonged by repeated administration of ACE inhibitors (15, 23, 24). Vascular tissue contains the components necessary to generate A-II locally (15, 25, 26), and it is known that local vascular formation of A-II is elevated in SHR (26) and 2K, 1C-RHR (27). It has been demonstrated that ACE inhibitors block sympathetic functions in SHR via an effect specific for the vasculature and linked to the vascular renin angiotensin system (21, 22, 28, 29). Thus, inhibition of local generation of A-II might explain the reduction in blood pressure caused by CV-5975 and enalapril in SHR and 2K, 1C-RHR. In SHR, the antihypertensive effect of CV-5975 was intensified by combining it with a diuretic, hydrochlorothiazide. Diuretic-induced sodium deficiency increases plasma renin level and the renin angiotensin system is important for blood pressure control in sodium depletion (30).

In 1K, 1C-RHR, the plasma renin level is normal and hypertension is thought to be maintained by renin-independent mechanisms (17, 30). The arterial wall renin concentration and ACE activity were significantly higher in 1K, 1C-RHR than in normotensive rats, but the values of plasma renin in 1K, 1C-RHR were lower than those in 2K, 1C-RHR (Table 2). Enalapril at 10 mg/kg, a dose sufficient for antihypertensive action in 2K, 1C-RHR and SHR and for inhibiting A-II-induced pressor response in normotensive rats, failed to reduce blood pressure in 1K, 1C-RHR. We have reported that high doses of captopril and delapril fail to induce antihypertensive activity in 1K, 1C-RHR (13). Moreover, reductions in blood pressure in 1K, 1C-RHR were not obtained after several days of dosing with an ACE inhibitor (4, 31). These findings suggest that the renin angiotensin system in vascular tissues plays little part in maintaining hypertension in 1K, 1C-RHR. In 1K, 1C-RHR, a single administration of CV-5975 dose-dependently reduced blood pressure (Fig. 7), indicating that the activity
of CV-5975 is qualitatively different to that of enalapril and captopril.

DOCA/salt hypertension is thought to be a low renin model of hypertension (32, 33). Although most ACE inhibitors failed to reduce blood pressure in this model (34, 35), CV-5975 reduced blood pressure. The levels of plasma and arterial wall renin were suppressed, although ACE activity was not reduced in plasma and aortae. In contrast to CV-5975, enalapril did not reduce blood pressure. In DOCA/salt hypertensive rats, augmentation of antihypertensive action, which was observed in SHR, was not observed by repeated administration of CV-5975 (Fig. 4). The vascular renin angiotensin system fails to accelerate in DOCA/salt hypertension, not like SHR. These findings indicate that the antihypertensive mechanism of CV-5975 may not involve the inhibition of the ACE activity of vascular tissue in this rat.

The degree of blood pressure reduction is correlated with the concentration of plasma and aortic wall renin before administration begins, greater reduction in blood pressure being observed in high renin hypertensive models. The fact that the antihypertensive activity of ACE inhibitors is seen at doses that block the A-I induced pressor response suggests that the antihypertensive properties of the inhibitor in models of renin-dependent hypertension are related to the ability of the compound to inhibit ACE activity. In experiments conducted in vitro, CV-5975 at $10^{-5}$ M had no effect on the contractile response of rat or rabbit aorta preparations induced by barium and tetraethylammonium, potassium or norepinephrine (data not shown). CV-5975 was not considered to possess the ability of directly relaxing the vascular tissue or to have Ca channel blocking and alpha-receptor blocking actions. Thus, a slight increase in heart rate observed in 2K, 1C-RHD treated with CV-5975 (1 mg/kg, p.o.) (Fig. 5) was considered not to be due to reflex tachycardia. The only mode of action of compounds such as enalapril and captopril appears to be inhibition of ACE activity, but their antihypertensive mechanisms are complicated and are not totally clarified as yet. CV-5975 is considered to exert the majority of its pharmacologic effects through a mechanism other than inhibition of ACE activity in renin-independent models of hypertension like 1K, 1C-RHR and DOCA/salt hypertensive rats.

In conclusion, CV-5975 is a potent and long-lasting antihypertensive agent that is effective in most forms of hypertension. Its action is mediated primarily through the inhibition of ACE activity and partly by a mechanism(s) that has not been elucidated.

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