Peripheral vs. Central Blockade of the Renin-Angiotensin System in Spontaneously Hypertensive Rats: Comparison of Novel AT1 Receptor Antagonist TCV-116 with Angiotensin Converting Enzyme Inhibitor Delapril

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In the present study, we evaluated the hemodynamic and metabolic profiles derived from oral administration of the angiotensin converting enzyme inhibitor, delapril 50 mg/kg/day, and the non-peptide angiotensin II type I (AT1) receptor antagonist, TCV-116 1 mg/kg/day, for five days, to try to discriminate AT1 receptor-related responses from the depressor properties of chronic treatment with delapril in spontaneously hypertensive rats (SHRs). Both TCV-116 and delapril oral administrations significantly decreased blood pressure without any changes in heart rate. Delapril induced dipsogenic response and natriuresis associated with augmentation of urinary catecholamine excretion, while TCV-116 did not cause any changes in these variables. Neither delapril nor TCV-116 changed urinary excretion of prostaglandin (PG) E2 and 6-keto PG F1α. We also examined the effects of centrally administered angiotensin converting enzyme inhibitor and AT1 receptor antagonist to determine the central role of these drugs. Either the active metabolite of delapril, delapril-M1 1 mg/kg/day, or the active form of TCV-116, CV-11974 0.1 mg/kg/day, were administered intracerebroventricularly for five days. Both treatments significantly decreased the blood pressure, in association with augmentation of the baroreceptor reflex control of heart rate, in response to phenylephrine injection. These findings suggest that the depressor properties of orally administered delapril are more complex than those of TCV-116, while central blockade by both angiotensin converting enzyme and AT1 receptor decreases blood pressure in part through baroreceptor sensitization. (Hypertens Res; 1993 16: 239–246)

Key Words: AT1 receptor antagonist, sodium balance, sympathetic nervous system, prostaglandin, baroreceptor reflex

Angiotensin converting enzyme inhibitors (ACEIs) have become one of the most popular medications for the treatment of hypertension, potentially offering favorable characteristics such as renal protection, beneficial effect on cardiac ischemia, and prevention of vascular proliferation (1). However, the precise mechanism underlying the depressor properties of ACEIs is not completely understood. Inhibition of the generation of circulating Ang II in plasma is proposed to be the primary mechanism for their depressor action (2). However, ACEIs possess other actions, such as bradykinin accumulation, to augment the depressor systems (3, 4). Blockade of the tissue renin-angiotensin system (RAS) is another mechanism to explain in part the depressor effect evoked by chronic administration of ACEIs (5–7).

The central nervous system is one of the tissues in which increased activity of the tissue RAS plays an important role in the pathogenesis of hypertension (8, 9). In fact, spontaneously hypertensive rats (SHRs) are reported to be characterized by abnormal augmentation of the brain RAS (9), and selective inhibition of brain Ang II with either ACEI or [Sar1, Ala8]-Ang II normalizes blood pressure (9). However, the question of whether systemically administered ACEIs can reach the central nervous system across the blood-brain barrier (BBB) and elicit their central effect to decrease the blood pressure still requires further investigation (9).

Recently, the development of specific non-peptide Ang II receptor antagonists has made it possible to
differentiate the physiological properties of the Ang II (AT₁) and AT₂ receptor-mediated responses of Ang II (10,11). Considerable evidence has been accumulated to suggest that AT₁ receptor-mediated responses play dominant roles in vasoconstriction and in aldosterone release induced by Ang II (12), and AT₁ receptor blockade successfully decreases blood pressure in renin-dependent hypertensive models (10). One of the advantages of using these non-peptide receptor antagonists is that they lack the agonistic actions observed with peptide Ang II analogues such as [SAl-Ala³]-Ang II (10). By taking advantage of this, we can clearly isolate the physiological role of Ang II from other effects associated with ACEI treatment.

With this information in mind, we compared the hemodynamic and metabolic profiles of chronic administration of ACEI with those of an AT₁ receptor antagonist in SHRs to try to separate the physiological role of blocking the AT₁ receptor from other factors associated with ACEI treatment. Either delapril, an ACEI, or TCV-116, a novel non-peptide AT₁ receptor antagonist, was orally administered for five days, and changes in hemodynamics, humoral variables, and sodium and water balance were determined. We also evaluated the effects of central blockade of the RAS by ACEI and AT₁ receptor antagonist to elucidate the central roles of these compounds.

Materials and Methods

Experiments were performed in 12-week-old male SHRs obtained from Charles River Japan, Inc. (Atsugi, Kanagawa, Japan). Delapril, a prodrug form of angiotensin converting enzyme inhibitor, and its active metabolite, delapril-M1, and TCV-116, a selective AT₁ receptor antagonist, and its active metabolite, CV-11974, were kindly provided by Takeda Chemical Industries (Osaka, Japan). Delapril-M1 and CV-11974, were dissolved in saline and administered for five days, and changes in hemodynamics, humoral variables, and sodium and water balance were determined. We also evaluated the effects of central blockade of the RAS by ACEI and AT₁ receptor antagonist to elucidate the central roles of these compounds.

Metabolism Profile Determination

Twelve-week-old male SHRs were placed individually in metabolic cages in a room maintained at constant temperature (22°C) with a 12-h light-dark cycle. Rats were allowed free access to water and food intake, urinary volume and urinary sodium excretion were determined twice before and on the 1st, 2nd, 3rd, and 4th days of treatment. After obtaining basal values, rats were randomly divided into six groups: Group I, vehicle control PO administration (n = 7); Group II, delapril PO administration (50 mg/kg/day) (n = 7); Group III, TCV-116 PO administration (1 mg/kg/day) (n = 7); Group IV, vehicle control intracerebroventricular administration (ICV) (n = 7); Group V, ICV administration of M1 (active metabolite of delapril, 1 mg/kg/day) (n = 8); Group VI, ICV administration of CV-11974 (active metabolite of TCV-116, 0.1 mg/kg/day) (n = 8). Delapril and TCV-116 were administered PO by dissolving the drugs in drinking water (tap water). Delapril-M1 and CV-11974 were dissolved in saline and pH was adjusted to 8 by sodium bicarbonate. ICV administration was performed using an osmotic minipump (Alza, Palo Alto, CA, U.S.A.) at a flow rate of 1 μl/h. The systolic blood pressure was recorded by a tail plethysmographic technique on the day before and the 1st and the 3rd days of the therapy. Urine specimens on the 4th day and 5th day were also collected for the determination of urinary catecholamines and prostaglandins in PO treated groups.

Surgical Procedure

IVC Administration

Four days before the initiation of IVC drug administration, an IVC catheter was implanted in the lateral ventricle of rats under pentobarbital anesthesia (50 mg/kg, i.p.) as described previously (15). In brief, a 27-gauge stainless steel needle was bent at a right angle; one end was connected to a polyethylene tube, while the beveled-end was inserted into the lateral cerebral ventricle using the following coordinates: 0.8 mm posterior and 1.5 mm lateral to the bregma, and 3.8 mm ventral to the surface of the skull. The catheter was fixed to the skull with dental cement and a jeweler’s screw. The polyethylene end of the catheter was advanced into the intercapsular region subcutaneously and connected to an osmotic minipump. The solution was delivered into the lateral ventricle at a flow rate of 1 μl/h. On the day of the initiation of IVC drug administration, each pump was exchanged with a new one containing either delapril-M1 or CV-11974, under light ether anesthesia. The correct placement of the IVC catheter was confirmed by injection of dye in each rat after the experiment.

Hemodynamic Measurements

Direct measurements of mean arterial pressure (MAP) and heart rate (HR) were performed via a plastic catheter inserted into the right femoral artery under light ether anesthesia on the 4th day after the initiation of the treatment. On the following day, between 9:00 AM and noon, arterial pressure was recorded with a solid strain transducer (TP-400T, Nihon Kohden, Tokyo, Japan) in conscious freely moving animals. Hemodynamic variables were recorded on a multi-channel polygraph (RM-6000 series, Nihon Kohden).

Blood samples were withdrawn through the arterial catheter for the determination of plasma renin activity (PRA), plasma aldosterone concentration (PAC) and plasma Ang II immunoreactivity.
Biochemical Determination
For PRA and PAC determination, 1 ml of blood was collected in a prechilled tube containing 50 μl of 0.5 M disodium-ethylenediaminetetraacetic acid (Na₂-EDTA). For plasma Ang II determination, 2 ml of blood was collected in a prechilled tube containing 100 μl of the inhibitor cocktail solution: 25 mM Na₂-EDTA (final concentration in blood collecting tube), 0.44 mM o-phenanthroline, 0.12 mM pepstatin A, and 1 mM 4-(chloromercuri) benzoic acid (I₆). After centrifugation at 4 °C, plasma was frozen on dry ice and stored at −20 °C until assay. Ang II was concentrated on an Amprep C₈ mini-column (Amer sham, Aylesbury, U.K.) and measured using a specific radioimmunoassay (RIA) (I⁷) (antibody was kindly provided by Dr. Kazuaki Shimamoto, Sapporo Medical College, Sapporo, Japan). PRA and PAC were measured with commercially available RIA kits (Dainabot, Tokyo, Japan). Urine samples for measurement of catecholamines and prostanoids were collected in the presence of 6N HCl and 25 mM Na₂-EDTA, respectively. After measurement of urine volume, urine samples were stored at −20 °C until assay. Nor-epinephrine, epinephrine and dopamine were separated by HPLC and quantified by UV absorbance (I⁸). The intra-assay coefficients of variations (CV) were 4.4, 2.6, and 8.2% for epinephrine, nor-epinephrine, and dopamine, respectively. The inter-assay CV were 7.8, 5.5, and 7.7% for epinephrine, norepinephrine, and dopamine, respectively. Urinary prostaglandin E₂ and 6-keto-PG-F₁α were measured using commercial kits (Dade, Baxter Travenol Diagnostics Inc., Cambridge, MA, U.S.A.).

Baroreceptor Reflex Control of Heart Rate
In separate rats, the effect of administration of AT₁ receptor antagonist or ACEI on the baroreceptor reflex control of HR was investigated in each treatment group (n = 5 for each group). SHRs were treated with the same doses of drugs administered in the metabolic study by either the PO or ICV route for 5 days. On the 4th day of treatment, under light ether anesthesia, polyethylene catheters were placed in the abdominal aorta and inferior vena cava via the femoral artery and vein for measurement of hemodynamic changes and administration of drugs, respectively. The free end of each catheter was exteriorized in the interscapular region. Twenty-four hours later, the baroreceptor control of HR was studied in conscious freely moving animals. Rats were allowed to stabilize for at least 30 minutes. Changes in HR in response to pressor response elicited by graded doses of phenylephrine (PE 15, 30, 150, 300 μg/kg, Sigma) as well as depressor response induced by nitroglycerin (NTG 15, 30, 150, 300 μg/kg, Sigma) were monitored. The sensitivity of the baroreceptor reflex was calculated in every animal as the slope of the least-squares regression line for the change in MAP and HR elicited by drug administration. Baroreflex sensitivity induced by phenylephrine injection was analyzed separately from that induced by NTG injection. NTG was used to elicit depressor response as a non-specific vasodilator (I⁹).

Statistical Analysis
All values are expressed as mean ± SEM. The differences in systolic blood pressure and metabolic profiles among the PO and ICV treated groups were analyzed by two-way analysis of variance with repeated measures followed by Duncan’s multiple range test. The changes in hemodynamics and humoral variables with treatment were analyzed by one-way analysis of variance followed by Duncan’s multiple range test. Difference in baroreceptor sensitivity was evaluated by two-way analysis of variance using covariance followed by Duncan’s multiple range test. The criterion for statistical significance was p < 0.05.

Results
Effect of RAS Blockade on Hemodynamics
Figure 1 shows the time course of systolic arterial pressure recorded in SHRs treated by either PO or ICV drug administration. PO administration of either delapril or TCV-116 for 5 days caused a significant decrease in systolic blood pressure. Central blockade of either ACE or AT₁ receptor by ICV administration of delapril-M1 or CV-11974 caused an even greater decrease in systolic blood pressure with the doses used in this experiment. The MAP obtained by direct measurement via an indwelling catheter on the 5th day confirmed the depressor effects of both ACE inhibitor and AT₁ receptor antagonist compared with vehicle control in PO treated SHRs (154 ± 4, 132 ± 6 and 121 ± 4 mmHg for vehicle, delapril and TCV-116, respectively, F(2,18) = 15.17, p < 0.05) and also in ICV treated SHRs (152 ± 4, 114 ± 2 and 122 ± 2 mmHg for vehicle, delapril and TCV-116, respectively, F(2,18) = 51.87, p < 0.05). However, both ACE and AT₁ blockade did not change HR in either PO treated (372 ± 11, 369 ± 10 and 384 ± 10 beats/min for vehicle delapril and TCV-116, respectively) or ICV treated SHRs (380 ± 12, 386 ± 4 and 392 ± 9 beats/min for vehicle, delapril M-1 and CV-11974, respectively).

Effect of RAS Blockade on Water and Sodium Balance
Figure 2 illustrates the metabolic profiles in PO treated SHRs. PO administration of ACEI significantly increased water intake, whereas this diuretic effect was not observed in TCV-116 PO treated SHRs. The net water balance in delapril PO treated SHRs showed a positive balance early in the course of treatment. Significant augmentation of sodium excretion after PO treatment with delapril resulted in a negative balance of sodium throughout the experiment. On the other hand, TCV-116 PO treatment did not show any effects on water and sodium balance. ICV administration of ACEI or AT₁ receptor antagonist did not cause any changes in either water or sodium balance compared with vehicle-treated SHRs (Fig. 3).
Effect of RAS Blockade on Humoral Factors

Table 1 summarizes humoral values in PO treated groups. Both TCV-116 and delapril significantly increased PRA, and TCV-116 significantly increased plasma levels of Ang II. The 24-h urinary epinephrine and norepinephrine excretion measured between the 3rd and 4th day of treatment did not differ between the vehicle-treated SHRs and ACEI or AT1 receptor antagonist treated SHRs. Urinary excretion of dopamine was significantly increased in ACEI PO treated SHRs compared to vehicle control.

**Fig. 1.** Upper: Changes in systolic blood pressure with oral administration (PO) of vehicle (Veh, n=7), angiotensin type 1 receptor antagonist (AT1-Ant, n=7), or angiotensin converting enzyme inhibitor (ACEI, n=7). Bottom: Changes in systolic blood pressure with intracerebroventricular administration (ICV) of vehicle (n = 7), angiotensin type 1 receptor antagonist (n = 8), or angiotensin converting enzyme inhibitor (n = 8). *p<0.01 compared with vehicle control. With both PO and ICV administrations, there were no differences in systolic blood pressure between AT1-Ant and ACEI treated SHRs.

**Fig. 2.** Metabolic profiles in SHRs orally administered vehicle (n = 7), angiotensin type 1 receptor antagonist, TCV-116 (AT1-Ant, n = 7) or angiotensin converting enzyme inhibitor, delapril (ACEI, n = 7). Figures show changes in water intake, urine volume, water balance, urinary sodium excretion, and sodium balance. *p<0.01 compared with vehicle control.
There was also no statistical difference in urinary excretion of prostaglandin E2 and 6-keto PGF1α among the PO treatment groups. Table 2 summarizes plasma values in ICV treated groups. Both AT1 receptor antagonist and ACEI significantly increased PRA, resulting in increase in plasma level of Ang II.

Discussion

Recent development of specific non-peptide AT1 receptor antagonists has led to further understanding of the pathophysiological role of the renin angiotensin system in the genesis and maintenance of hypertension. Many lines of evidence have been gathering to suggest that the vasoconstriction and aldosterone release of Ang II are AT1 receptor mediated properties (12). Since ACEIs possess many features in addition to blockade of the RAS (4), we tried to focus on the difference between the effects of ACEI and AT1 receptor antagonist on water and sodium balance, the sympathetic nervous system, and prostaglandins. Although both peripheral AT1 receptor blockade and ACE inhibition showed similar effects on hemodynamic, urinary PG excretion, and the baroreflex sensitivity of HR control, the ACEI showed quite different effects on water and sodium metabolism as compared with the selective AT1 receptor antagonist. PO administration of delapril significantly increased urinary excretion of sodium, resulting in a negative sodium balance throughout the experimental period. This natriuresis was associated with an increase in urinary catecholamine excretion. On the other hand, PO administration of the non-peptide AT1 receptor antagonist decreased blood pressure without changing water and sodium balance.

Blockade of the RAS has been shown to antagonize the facilitatory action of Ang II on noradrenaline release (20). In the present study, PO administration of TCV-116 did not change urinary excretion of catecholamines, an index of activity of the sympathetic nervous system. However, in view of reduced blood pressure, this finding may reflect the suppression of the sympathetic nervous system. In fact, urinary excretion of catecholamines was elevated in delapril PO treated SHR, in accordance with another study (21). We also observed an increase in dopamine excretion in response to delapril administration. Since activation of renal tubular dopaminergic system results in inhibition of sodium reabsorption (22), the natriuresis observed in delapril PO treated SHRs may have been partly due to its dopaminergic activation in the kidney. Further study will be necessary to elucidate this possibility since the dopaminergic augmentation is not always observed (21).

The activation of the prostaglandin system is another mechanism accompanying ACEI treatment (23). However, in the present study, urinary excretion of prostaglandins was not augmented in ACEI
Although the involvement of the prostaglandin system is proposed for captopril administration both in experimental and clinical conditions, an increase in urinary excretion of prostaglandins was not always demonstrated, especially in compounds without the SH-residue (23).

Compared with ACEI, AT1 receptor antagonist decreased blood pressure to a similar extent without changing mineral-fluid balance by oral administration. These findings indicate that hypotensive response induced by ACEI is mainly due to the elimination of AT1 receptor mediated properties.

Table 1. Effect of Oral Administration of AT1-antagonist and Angiotensin Converting Enzyme Inhibitor on Humoral Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Vehicle</th>
<th>AT1-Ant</th>
<th>ACEI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>7</td>
<td>7</td>
<td>7</td>
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<tr>
<td>Plasma Renin Activity (ng/ml/h)</td>
<td>1.0 ± 0.2</td>
<td>7.5 ± 0.7**</td>
<td>5.8 ± 0.7**</td>
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<td>Plasma Ald Concentration (pg/ml)</td>
<td>199 ± 55</td>
<td>240 ± 86</td>
<td>248 ± 65</td>
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<tr>
<td>Plasma Angiotensin II (pg/ml)</td>
<td>19.6 ± 0.5</td>
<td>36.7 ± 3.9*</td>
<td>21.4 ± 2.1</td>
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<td>Urinary PG E2 (ng/24 h)</td>
<td>65 ± 13</td>
<td>96 ± 16</td>
<td>89 ± 36</td>
</tr>
<tr>
<td>Urinary 6-keto-PGF1α (ng/24 h)</td>
<td>15.0 ± 3.4</td>
<td>18.5 ± 3.1</td>
<td>20.1 ± 2.4</td>
</tr>
<tr>
<td>Urinary Norepinephrine (ng/24 h)</td>
<td>154 ± 38</td>
<td>291 ± 59</td>
<td>371 ± 120</td>
</tr>
<tr>
<td>Urinary Epinephrine (ng/24 h)</td>
<td>22.7 ± 7.3</td>
<td>21.3 ± 6.0</td>
<td>49.8 ± 5.1*</td>
</tr>
<tr>
<td>Urinary Dopamine (µg/24 h)</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>3.5 ± 1.1*</td>
</tr>
</tbody>
</table>

Humoral variables in SHRs orally administered vehicle, angiotensin type 1 receptor antagonist (AT1-Ant), or angiotensin converting enzyme inhibitor (ACEI). Data are mean ± SEM. *p < 0.05, **p < 0.01 compared with vehicle-treated rats, by one-way analysis of variance followed by Duncan's multiple range test. Ald, aldosterone; PG, prostaglandin.

Table 2. Effect of Central Administration of AT1-antagonist and Angiotensin Converting Enzyme Inhibitor on Humoral Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Vehicle</th>
<th>AT1-Ant</th>
<th>ACEI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>7</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Plasma Renin Activity (ng/ml/h)</td>
<td>1.4 ± 0.2</td>
<td>8.2 ± 1.0**</td>
<td>6.1 ± 0.8**</td>
</tr>
<tr>
<td>Plasma Ald Concentration (pg/ml)</td>
<td>292 ± 192</td>
<td>388 ± 102</td>
<td>283 ± 117</td>
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<tr>
<td>Plasma Angiotensin II (pg/ml)</td>
<td>14.3 ± 0.5</td>
<td>28.1 ± 3.2*</td>
<td>27.2 ± 2.2*</td>
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</table>

Humoral variables in SHRs centrally administered vehicle, angiotensin type 1 receptor antagonist (AT1-Ant), or angiotensin converting enzyme inhibitor (ACEI). Data are mean ± SEM. *p < 0.05, **p < 0.01 compared with vehicle-treated rats, by one-way analysis of variance followed by Duncan's multiple range test. Ald, aldosterone.

We observed that the net water balance was positive in delapril treated SHR during the early phase of the treatment. This effect of ACEI is known as "paradoxical dipsogenic reaction" (24). Since central ACE inhibition depends on the dose of inhibitor given by systemic administration (9), partial inhibition of ACE activity in the brain allowed circulating Ang I to change to Ang II inside the BBB to stimulate drinking. The finding that peripheral AT1 blockade did not increase water consumption in
SHRs despite an increase in plasma Ang II indicates that this compound may access the central nervous system and block the AT₁ receptor in the brain like another AT₁ receptor antagonist, losartan (25).

Although the possible participation of the AT₂ receptor in vasopressin release, prostaglandin synthesis, cell growth or development has been advocated recently (26, 27), little is known about the physiological roles of the AT₂ receptor compared with the AT₁ receptor. Since AT₂ receptor is relatively abundant in certain areas in the brain (28) and the selective AT₁ blockade could stimulate AT₂ receptor, AT₂ receptor activation may contribute to the differences between ACEI and AT₁ receptor antagonist.

Baroreflex sensitivity was significantly augmented in both AT₁ receptor antagonist and ACEI ICV treated SHRs. These findings are consistent with a previous study demonstrating that brain RAS blockade increased baroreflex sensitivity in SHRs (29, 30). In rat brain, the population of AT₁ receptors is concentrated in regions closely related to blood pressure regulation and the baroreceptor reflex system, such as the nucleus of the solitary tract, paraventricular nucleus, area postrema and subfornical organ (28). These findings together agree with the concept that the brain RAS is augmented in SHRs and contributes to hypertension through activation of the sympathetic nervous system and attenuation of baroreflex function (9).

In conclusion, oral administration of either ACEI or AT₁ receptor antagonist decreased blood pressure similarly. However, their effects on water and sodium metabolism were quite different, which may be due partly to renal dopaminergic involvement or their accessibility to the brain. Selective blockade of either ACE or AT₁ receptors in the central nervous system decreased blood pressure in association with augmentation of baroreflex sensitivity, without changing water or sodium balance. These findings indicate that elimination of AT₁ receptor mediated-responses plays dominant roles in the depressor action of ACEI treatment both peripherally and centrally; however, ACEIs also possess other actions, especially on water and sodium homeostasis, when administered systemically.

References

5. Cushman DW, Wang FL, Harvey CM, Deforrest JM: Differentiation of angiotensin-converting enzyme


