Role of the Tissue Renin-Angiotensin System in the Action of Angiotensin-Converting Enzyme Inhibitors (43867)

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Abstract. **The mechanism of the blood pressure-lowering action of chronic administration of angiotensin-converting enzyme (ACE) inhibitors is still controversial. We investigated the effects of the ACE inhibitors, captopril and perindopril, on the renin-angiotensin system (RAS) in plasma and tissues (adrenal gland and kidney) in the rat. Captopril or perindopril was infused intraperitoneally into rats via a mini-osmotic pump for 6 days at a rate of 0.5 or 0.25 mg/kg/hr, respectively. Perindopril markedly increased plasma renin concentration (PRC) from 12.7 ± 1.1 to 867 ± 59 ng Ang I/ml/hr and significantly inhibited plasma angiotensin II (Ang II) from 17.5 ± 3.5 to 7.8 ± 0.6 pg/ml and plasma ACE activity from 31.6 ± 1.7 to 1.7 ± 0.3 U/liter. Captopril also increased PRC from 12.1 ± 2.1 to 147 ± 17 ng Ang I/ml/hr. However, it did not inhibit plasma Ang II (20.6 ± 1.9 vs 22.0 ± 2.1 pg/ml, N.S.) and increased plasma ACE activity from 35.9 ± 1.8 to 65.0 ± 4.8 U/liter. Perindopril increased kidney renin from 625.3 ± 84.6 to 2152.3 ± 233.4 μg/g/hr, while captopril produced a modest but insignificant rise in kidney renin (708.0 ± 107.1 vs 1083.3 ± 155.5 μg Ang I/g/hr, N.S.). On the other hand, both captopril and perindopril decreased adrenal Ang II significantly (from 21.1 ± 2.7 to 9.2 ± 0.5 pg/capsule and from 15.5 ± 2.9 to 2.0 ± 0.6 pg/capsule, respectively). Adrenal renin was not altered by either treatment. In spite of no inhibition of plasma Ang II, the pressor response to intravenous Ang I was still suppressed after captopril treatment. Both captopril and perindopril lowered the blood pressure of the rats significantly. Our results support the hypothesis that inhibition of tissue RAS is important for the hypotensive action of ACE inhibition.** [P.S.E.B.M. 1995, Vol 208]

The renin-angiotensin system (RAS) plays an important role as a modulator of blood pressure and as a regulator of various hormones to maintain volume homeostasis (1). In acute experiments, angiotensin-converting enzyme (ACE) inhibitors lower blood pressure, and this action is associated with inhibition of plasma ACE and lowering of plasma angiotensin II (Ang II). However, a dissociation between the hypotensive effect and the suppression of plasma RAS has been observed by other investigators (2–6) after chronic administration of ACE inhibitors. Unger *et al.* (7) reported that the prolonged antihypertensive action of ACE inhibitors may be related to persistent ACE inhibition in tissues. In the present study, we investigated the role of the tissue renin-angiotensin system in the action of ACE inhibitors. We measured the blood pressure and the components of the RAS after chronic ACE inhibitor treatment in plasma and the adrenal gland using two different ACE inhibitors, captopril and perindopril. The adrenal gland was selected because it has been generally accepted that the adrenal contains all components of the RAS (i.e., renin and its mRNA [8–10], angiotensinogen mRNA [11], Ang II [12–15], and ACE [16–17]).

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Materials and Methods

Male Sprague-Dawley rats (200–250 g) were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Rats were housed under a 12:12-hr light:dark cycle and provided with water and diet containing normal sodium (Wayne Lab Blox, Chicago, IL) *ad libitum*. All surgical procedures were reviewed and approved by the Institutional Animal Care and Use Committee.

Chronic ACE Inhibitors Treatment. All rats were implanted with mini-osmotic pumps (ALZA Corp., Palo Alto, CA) containing 0.9% saline or the ACE inhibitor, captopril or perindoprilate (Institute de Recherches Internationales Servier & Compagnie-Development, Courbevoie Cedex, France), under light ether anesthesia. Captopril and perindoprilate were infused intraperitoneally at the rate of 0.5 and 0.25 mg/kg/hr, respectively.

After 5 days of infusion, the blood pressure was measured by the tail cuff method on conscious but restrained rats. After measuring blood pressure, all rats were inserted with a polyethylene tubing (PE 50) into the carotid artery under ether anesthesia. On the next day, blood was collected from conscious rats through the carotid catheter into chilled tubes containing Na_2EDTA (13.4 m*M*, final concentration), pepstatin A (7 n*M*) and captopril (10 μ*M*) for the measurement of plasma Ang II. Another blood sample was collected into chilled tubes containing Na_2EDTA (4 m*M*) for the measurement of plasma renin concentration (PRC), and into heparinized tubes for the measurement of plasma ACE activity. Plasma ACE activity was measured on fresh unfrozen plasma by spectrophotometric assay (18) using hippuryl-histidyl-leucine (Sigma Chemical Co., St. Louis, MO) as substrate. The rats were then killed by decapitation, and the adrenal glands were removed after flushing with cold 0.9% saline through the aorta. The capsular portions (capsules-glomerulosa cell layer) were separated from the decapsular portions (fasciculata, reticularis, and medullary cell layer) and immediately frozen on acetone and dry ice. The left adrenal capsules were processed for the measurement of Ang II, and the right adrenal capsules were processed for renin activity. The kidney was also collected and rapidly frozen in liquid nitrogen, and used for the measurement of renin activity.

Pressor Response to Angiotensin I and II. In order to study the effect of chronic captopril treatment on ACE activity, the pressor response to intravenous Ang I and Ang II was investigated. After 6 days of captopril treatment, two polyethylene catheters were inserted into the carotid artery and the jugular vein under pentobarbital anesthesia. Arterial pressure in the anesthetized rats was monitored with a data recording system (Model 79 Polygraph; Grass Instrument Co., Quincy, MA). After the blood pressure stabilized, 100 μl of 0.9% saline was infused intravenously for the control response, and then 100 ng of Ang I dissolved in 100 μl of saline was administered, and the pressor response was recorded. Ang I was administered twice at 10-min intervals. Thereafter, the pressor response to Ang II (50 ng, dissolved in 100 μl of saline) was also measured.

Measurement of Plasma Renin Concentration. Plasma renin concentration (PRC) was measured by RIA of Ang I generated by plasma was exogenous renin substrate as we previously described (19). The renin substrate was plasma obtained from male SD rats 48 hr after nephrectomy. The Ang I generated was measured by RIA with RIANEN Angiotensin I RIA kit (New England Nuclear Research Products, Boston, MA).

Measurement of Plasma Angiotensin II. Immediately after collecting blood, the plasma was extracted with C^{18}-SepPak cartridge (Waters Chromatography division/Millipore Corporation, Milford, MA) using acetonitrile for elution. After evaporation, plasma Ang II was measured by the combination of HPLC and RIA. Briefly, samples were chromatographed by HPLC system (Waters Model 510) with a reversed-phase analytical column (Aquapore RP-300, 4.6 mm × 25 cm; Brownlee Labs Inc., Santa Clara, CA). Water and acetonitrile were used as solvents (77:23%). Fractions were collected every 1 min, evaporated with a gentle stream of air, and reconstituted with assay diluent (0.02% NaN_3 and 2.5% BSA in 0.2 *M* Trizma base, pH 7.4). The samples, ^{125}I-angiotensin II (New England Nuclear Research Products, Boston, MA) and rabbit anti-angiotensin II serum (IgG Corp., Nashville, TN) were incubated for 3 days at 4°C and the unbound tracer was pelleted by the addition of activated charcoal, followed by centrifugation at 3000 rpm at 4°C for 20 min.

Measurement of Adrenal Renin, Adrenal Angiotensin II, and Kidney Renin. Adrenal capsules were homogenized with a Teflon-glass homogenizer in 1.0 ml Trizma base (0.2 *M*, pH 7.4) containing 2×10^{-2} *M* Na_2EDTA, 1.5×10^{-5} *M* pepstatin A, and 10^{-5} *M* captopril. The homogenate was centrifuged at 1800*g*, 4°C for 30 min. The supernatant (500 μl) was extracted with a C^{18}-SepPak cartridge using acetonitrile as elution. These operations were completed within 2 hr after collecting the adrenal glands. After evaporation, the samples were reconstituted with assay diluent and adrenal Ang II was measured by RIA by the same method used for plasma Ang II. The mean recovery of unlabeled Ang II after extraction was 91.4% ± 4.6% (n = 5). The data are not corrected for recovery. In a previous publication we reported that over 90% of the adrenal Ang II

measured by our RIA is authentic Ang II by HPLC (15).

For the measurement of renin activity, the kidney was homogenized with ice cold Tris acetate buffer (0.1 μ, pH 7.4) containing 20 m*M* Na_2EDTA and 7.5 m*M* captopril after measuring wet tissue weight. After centrifugation at 1800 *g*, 4°C for 30 min, the supernatant was diluted with Tris acetate buffer (0.1 μ, pH 7.4) containing Na_2EDTA, captopril (7.5 μ*M*) and 5% BSA. Renin activity in the diluted kidney homogenate was measured by the same method used for the measurement of PRC.

Statistical Evaluation. Results are shown as the mean ± SEM. Student's *t* test was used for the statistical analysis of other data. Statistical significance was defined as a $P < 0.05$.

Results

Figure 1 and 2 depict the effects of chronic captopril or perindopril treatment on the renin-RAS in plasma and tissue. As expected, chronic perindopril treatment markedly increased PRC (from 12.7 ± 1.1 to 867.1 ± 58.7 ng Ang I/ml/hr, $P < 0.01$), and decreased plasma Ang II (from 17.5 ± 3.5 to 7.8 ± 0.6 pg/ml, $P < 0.05$). Chronic captopril treatment also significantly increased PRC (from 12.1 ± 2.1 to 146.5 ± 16.7 ng Ang I/ml/hr, $P < 0.01$); however, it did not decrease plasma Ang II (20.6 ± 1.9 vs 22.0 ± 2.1 pg/ml, N.S.). Perindopril increased kidney renin significantly from 625.3 ± 64.6 to 2152.3 ± 233.5 μg/g/hr, $P < 0.01$, while it did not increase adrenal renin (5.4 ± 0.8 vs 6.7 ± 1.4 ng Ang I/adrenal hr, N.S.). Captopril did not increase either kidney renin (708.0 ± 107.1 vs 1083.3 ± 155.5 μg/g/hr, N.S.) or adrenal renin (6.9 ± 0.7 vs 9.9 ± 1.0 ng Ang I/adrenal/hr, N.S.). In spite of no inhibition of plasma Ang II levels, captopril lowered the blood pressure of rats in a similar fashion to perindopril. Both captopril and perindopril decreased adrenal angiotensin II significantly from 21.1 ± 2.7 to 9.2 ± 0.5 pg/capsule, $P < 0.01$ and from 15.5 ± 2.9 to 2.0 ± 0.6 pg/capsule, $P < 0.01$, respectively. Perindopril decreased plasma ACE activity from 31.6 ± 1.7 to 1.7 ± 0.3 U/liter, $P < 0.01$. On the other hand, chronic captopril treatment increased plasma ACE activity significantly (from 35.9 ± 1.8 to 65.0 ± 4.8 U/liter, $P < 0.01$).

Table 1 outlines pressor response to angiotensin I and II. Administration of 100 μl of saline did not alter blood pressure. In spite of no suppression of plasma Ang II after captopril treatment, the pressor response to intravenous Ang I in the chronically captopril treated rats was decreased significantly (14.8 ± 4.3 mm Hg in the captopril group versus 30.8 ± 2.5 mm Hg in the control group, $P < 0.05$). The pressor re-

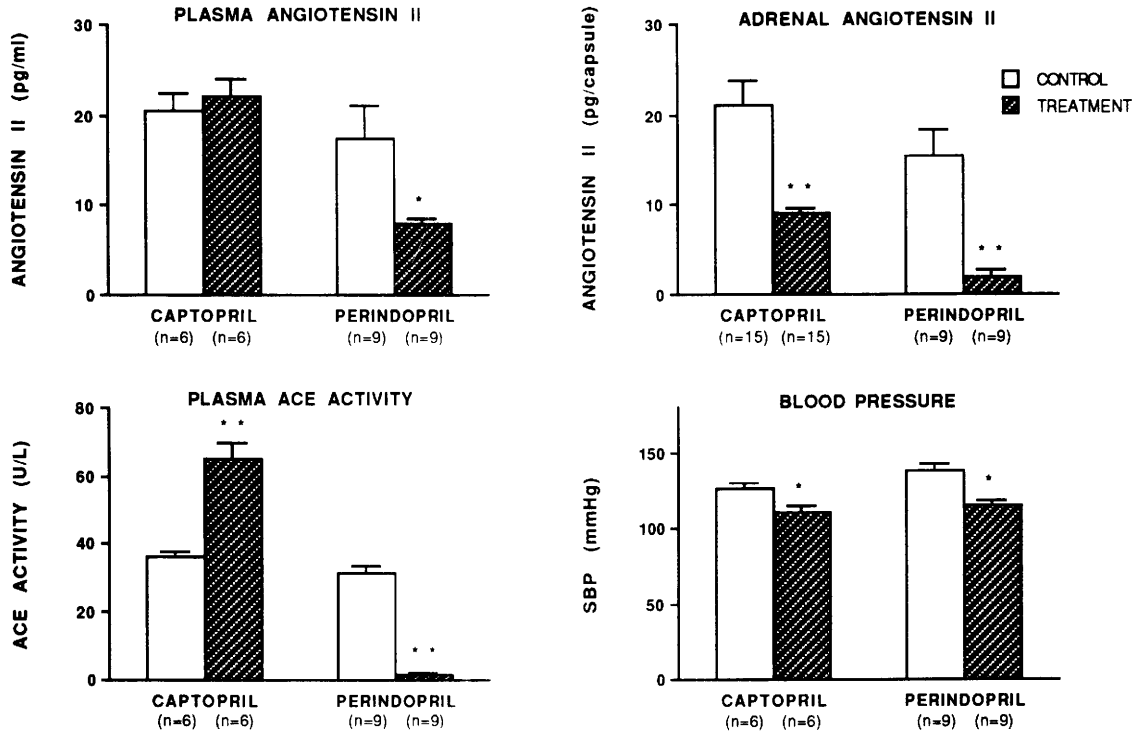

Figure 1. Plasma Ang II (left upper panel), adrenal Ang II (right upper panel), plasma ACE activity (left lower panel), and blood pressure (right lower panel) after chronic captopril or perindopril treatment. Open bars represent control values in each treatment; hatched bars represent the values after captopril or perindopril treatment; each bar represents the mean ± SEM. The number of rats is shown below each bar. *$P < 0.05$ compared with each control group; **$P < 0.01$ compared with each control group.

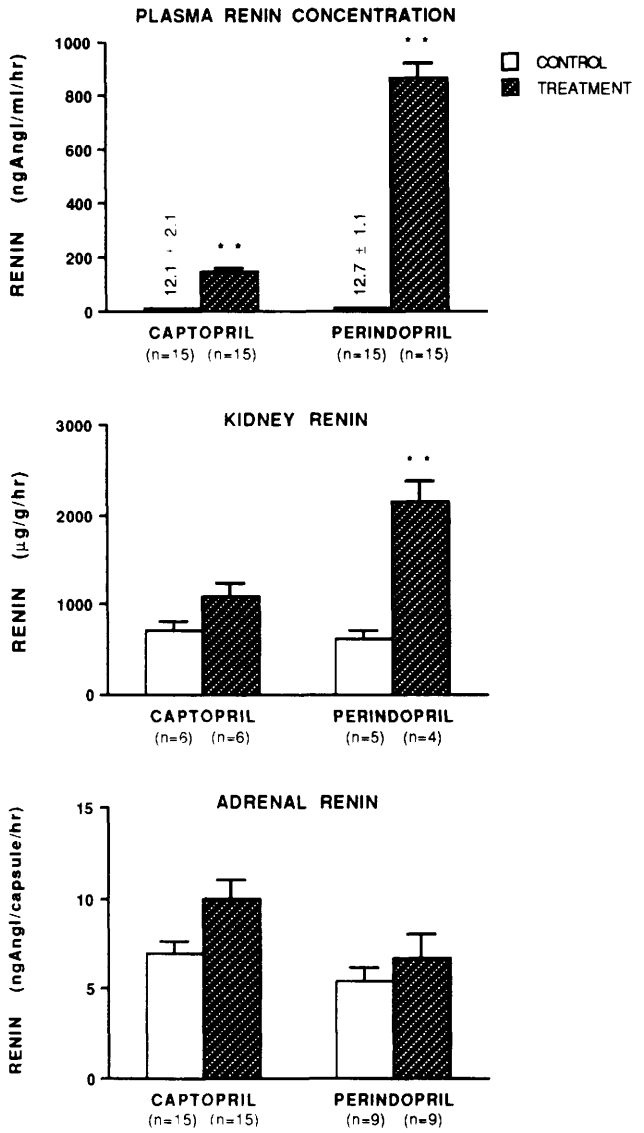

Figure 2. Plasma renin concentration (PRC), kidney renin, and adrenal renin activity after captopril or perindopril treatment. The data are shown as the mean ± SEM. The number of rats is shown below each bar. $^{**}P < 0.01$ compared with each control value.

sponse to intravenous Ang II was 33.0 ± 2.4 mm Hg in the control rats and 30.6 ± 6.3 mm Hg in the captopril treated rats (N.S.).

Discussion

Experimental evidence suggests that the peripheral tissues may be a major site of generation of Ang I and Ang II. In humans, Admiraal *et al.* (20) reported that 50%–90% of endogenous Ang I in veins was derived from regional *de novo* production. Recently, Mullins *et al.* developed the transgenic hypertensive rat TGR (mRen2)27 (21) in which renin synthesis is clearly expressed in a number of extra renal tissues. The traditional concept of the function of the renin

Table I. Pressor Response to Intravenous Ang I (100 ng) and II (50 ng) After Chronic Captopril Treatment

	Control	Captopril
Pressor response to Ang I (mm Hg)	30.8 ± 2.5 (n = 4)	14.8 ± 4.3[a] (n = 5)
Pressor response to Ang II (mm Hg)	33.0 ± 2.4 (n = 4)	30.6 ± 6.3 (n = 5)

Note. Ang I and II were administered to rats through the jugular vein catheter. Blood pressure was monitored via a carotid artery catheter. The data are shown as the mean ± SEM. n = 4: in the control group; n = 5: in the captopril-treated group.
[a] $P < 0.05$ compared with control.

angiotensin system as an endocrine system is, therefore, being challenged.

Acute administration of ACE inhibitors blocks the conversion of Ang I to Ang II. The hypotensive effect correlates with inhibition of Ang II formation. However, after chronic administration of ACE inhibitors, the mechanisms of the hypotensive action are still controversial. Biollaz *et al.* (2) reported that 12 to 16 hr following the intake of MK421, (enalapril) plasma Ang II levels returned to baseline, yet the hypotensive effect was still present in the hypertensive patients. Wilkes (3) noted that chronic ACE inhibition with MK 421 of sodium depleted rat resulted in Ang II levels higher than baseline. Similarly, Mento *et al.* (4) reported that 1 week or 2 months of treatment of rats with enalapril increased plasma Ang II levels significantly above baseline. Captopril administration via the drinking water for 3 weeks increased serum ACE activity in rats in a dose-dependent fashion (5). Also, Forslund *et al.* reported a dissociation between the inhibition of ACE activity and the hypotensive action in SHR rats during chronic administration of captopril. In short, several investigators have shown a discrepancy between the blood pressure-lowering effect of ACE inhibitors and the inhibition of the plasma renin-angiotensin system after chronic administration of ACE inhibitors.

Our data with chronic captopril treatment also showed no lowering of plasma Ang II, despite a lowering of the blood pressure and a reduction of the pressor effect of Ang I injections. Furthermore, our data showed a rise in serum ACE activity in spite of the chronic continuous infusion of captopril. Other investigators observed a similar increase in serum and plasma ACE activity in rats chronically treated with captopril (22, 23).

Chronic therapy with ACE inhibitors induces ACE enzyme production, but since captopril is a weaker ACE inhibitor than perindopril, the *in vitro* assay for ACE activity may not reflect the *in vivo* inhibitory action of captopril. However, despite the lack of reduction of plasma AII, chronic captopril therapy

lowered the blood pressure and blocked the pressor effect of AI injections. Captopril did reduce tissue AII concentration in the adrenal gland. These data would suggest that the hypotensive effect of ACE inhibition is due in part to their inhibition of tissue ACE. On the other hand, perindopril lowered both plasma and tissue AII but had a similar hypotensive effect as captopril.

Of further interest is the elevated PRC seen with captopril treatment despite a normal circulatory AII level. This finding suggests that inhibition of renal ACE may have occurred and lowered renal AII resulting in an increase in renin release. Although we did not measure renal AII, we did measure the content of AII in one tissue, the adrenal, and it was lowered. Campbell *et al.* (24) noted an increase in plasma renin in rats treated with oral perindopril, despite a failure to reduce plasma AII levels, while kidney AII level were markedly reduced. These data support the hypothesis that the tissue action of ACE inhibition are important.

Inhibitors of ACE have other actions besides the inhibition of the conversion of Ang I to Ang II, such as stimulation of prostaglandins (25–27), decrease kinin degradation (28–30), and interaction with catecholamines (31). Further investigations are necessary to clarify the contribution of these factors to the blood pressure-lowering effect of ACE inhibitors.

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