Converting Enzyme Inhibition During Chronic Angiotensin II Infusion in Rats
Evidence Against a Nonangiotensin Mechanism

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SUMMARY Interpretation of results obtained with angiotensin-converting enzyme inhibition in hypertensive patients has been obscured by the possibility of nonangiotensin-mediated mechanisms, particularly bradykinin potentiation. Using subcutaneous osmotic pumps to infuse angiotensin II chronically into conscious rats, we have compared the effects of converting enzyme inhibition (CEI) by oral captopril administration to those of dextrose. In this setting of constant angiotensin II levels, any apparent effects of CEI must be mediated by a nonangiotensin-related mechanism. Angiotensin II infusion at 30 ng/mln increased mean blood pressure by an average of 22 mm Hg. Following 7 days of CEI, effective blockade of converting enzyme was established both by a 10-fold elevation of vasodepressor sensitivity to exogenous bradykinin and a markedly decreased plasma converting enzyme activity. On the ninth day of angiotensin II infusion, mean arterial pressure, heart rate, and plasma renin activity were not different between the CEI and dextrose-treated groups. Similarly, blockade of angiotensin II by saralasin induced a comparable fall in blood pressure in both groups. Metabolic studies also revealed no long-term differences in water and food intake, weight change, or sodium and potassium metabolisms. These findings suggest that, in the continued presence of angiotensin II, there is no detectable hemodynamic or metabolic effect of chronic converting enzyme inhibition, and therefore that bradykinin plays little or no role in its long-term antihypertensive action. (Hypertension 3: 269-276, 1981)

KEY WORDS • chronic converting enzyme inhibition • acute and chronic angiotensin II infusion • bradykinin sensitivity • blood pressure regulation • electrolyte balance • plasma renin activity • plasma converting enzyme activity

RESULTS From studies with inhibitors of the renin-angiotensin system have suggested that angiotensin participates, at least to some degree, in many forms of hypertension in men and experimental animals. Moreover, it regulates the renal handling of sodium via its control of aldosterone secretion, modulation of renal blood flow, and possibly direct tubular effects. However, interpretation of the results from studies using such inhibitors as sar-1-ala-8-angiotensin II (saralasin or P 113), teprotide (SQ 20,881), and most recently captopril (SQ 14,225) has been obscured by some inherent properties of these drugs. Saralasin, a peripheral competitive antagonist of angiotensin II, has agonist properties of its own. Teprotide and captopril inhibit the generation of angiotensin II by blocking angiotensin-converting enzyme, which is identical with kininase II, a major route of degradation of bradykinin. Since the hemodynamic effects of bradykinin are opposite to those of angiotensin II and result in vasodilation, hypotension, and natriuresis, it has not been clear to what degree the effects of converting enzyme inhibition reflect a decrease in angiotensin II levels as opposed to increased activity of bradykinin.

The present study was designed to examine whether long-term converting enzyme inhibition (CEI) by captopril exerts any blood pressure (BP) lowering effect separate from reducing angiotensin II. For this purpose, hypertension was induced in rats by continuous long-term angiotensin II infusion.

Methods Male Wistar albino rats (Institut für biologisch-medizinische Forschung AG, Füllinsdorf, Switzerland) weighing between 210 and 330 g were used. All
were maintained on a normal rat chow diet (U.A.R., Geneva) containing 0.103 mEq/g sodium and 0.197 mEq/g potassium. Prior to the study, rats were taught to accept medication by gastric gavage twice daily.

Chronic Angiotensin II Infusion

Angiotensin II amide (Hypertensin, Ciba-Geigy, Basel, Switzerland) was infused chronically by miniature osmotic pumps (Alzet 1702, Alza Company, Palo Alto, California) placed subcutaneously. From the pump 10 cm of flexible siliconized catheter (Silastic, Dow Corning, Midland, Michigan) led to the jugular vein. To prevent peptide adhesion, pumps and catheter attachments were siliconized with 1% silicone (Siliclad, Dow Corning, Midland, Michigan) for 48 hours, then dried at 40°C for 24 hours. Angiotensin was dissolved in 0.9% saline to provide an infusion rate of approximately 30 ng/min (not corrected for possible impurities) at a nominal pump infusion rate of 0.5 μl/hr. To prevent blood reflux during long-term placement, the catheter tips were closed with silicone adhesive (Silastic medical adhesive, Dow Corning, Midland, Michigan), and slit valves were made in the side of the tubing with a 23 gauge needle just before placement. Twenty-one rats had osmotic pumps placed and were allowed to recover for 2 days. Thereafter, the synthetic, orally active angiotensin-converting enzyme inhibitor, D-2-methyl-3-mercapto-propanoyl-L-proline (SQ 14,225) or captopril, converting enzyme inhibitor, D-2-methyl-3-mercapto Propionyl-L-proline, was dissolved in 5% dextrose and diluted serially with saline. The response to a 0.1 ml during continuous BP and pulse recording, allowing 5 to 10 minutes between doses. A solution of synthetic bradykinin (Peptide Research Foundation, Beckman, Palo Alto, California), 6 μg/ml, was prepared from a single batch and frozen in small aliquots. Prior to use, an aliquot was thawed and diluted serially with saline. The response to a given dose was taken as the maximum drop in BP, invariably within 15 seconds of injection and preceding reflex heart rate changes. Four to six doses were used with care to obtain a range reaching a drop of at least 45–50 mm Hg. The same aliquot of bradykinin was used for the dextrose- and captopril-treated animals each day. The fall in MAP was plotted for each animal vs the dose of bradykinin on a logarithmic scale, and a linear regression plot obtained. As a numerical expression of sensitivity to bradykinin, the dose that produced a 30 mm drop was selected (dose - log/kg).

Saralasin Infusion

After a 1-hour recovery period, baseline values were again obtained. Then sar-1-ala-8-angiotensin II (Saralasin, Eaton, Norwich, New York) was infused at a rate of 5 μg/min for 30 minutes by syringe infusion pump (Sage instruments, M 355, Cambridge, Massachusetts) to confirm the presence of high circulating levels of angiotensin II. All animals used in the final analysis had a BP drop of 24 mm Hg or more; of the 24 animals started, three were finally excluded due to the finding of normal BP and no BP drop after saralasin, indicating pump malfunction. After stopping the saralasin infusion, a period of 2 hours was allowed for recovery. Just prior to sacrifice, 1.5 ml of blood was obtained over 15 seconds through the arterial catheter for the measurement of plasma renin activity (PRA) and catecholamines. Thereafter, 3 ml were taken for determination of plasma converting enzyme activity and sodium and potassium concentrations. These were placed at 0°C, centrifuged immediately, and separated for storage at -30°C. Catecholamine samples were stored at -70°C.

Metabolic Studies

Eight animals were housed individually in clear plastic metabolic cages providing separation of feces and urine (Ehret, Emmendingen, W. Germany). Food was presented as a paste in predetermined quantities, and any food remaining was redried and weighed daily. Supplemental water was provided in calibrated bottles, measured and refilled daily. Cages were rinsed carefully with distilled water to a final volume of 500 ml by the same two workers. Daily urinary sodium and potassium excretion were measured by flame photometry (Corning 455, Essex, England). After 1 week of habituation, baseline values for sodium and...
potassium balance and water intake were recorded for an additional week while the rats received dextrose gavage twice daily. Osmotic pumps containing angiotensin II were then placed and the studies continued for the protocol period described above. Half were treated by converting enzyme inhibition and half received dextrose by gavage starting on the second day following pump placement. Final testing was performed in an identical fashion to that of the other rats.

Acute Angiotensin II Infusion

For comparison to the chronic infusion experiments, five animals were catheterized in the manner used for final testing, with the exception of an additional venous cannula being placed in the left femoral vein. Two hours later, angiotensin II was infused by syringe infusion pump beginning at 30 ng/min and the BP and pulse recorded. Thereafter, angiotensin II infusion rates were increased to a final level of 300 ng/min.

Analytical Methods

Plasma renin activity was measured by radioimmunoassay. Plasma angiotensin-converting enzyme activity was determined by the radioenzymatic method using a radiolabelled acylated tripeptide as substrate (Ventrex Corporation, Portland, Maine). Plasma catecholamines were also quantitated by a radioenzymatic method.

Statistical Methods

Results are expressed as mean ± SEM. Differences between groups or responses were tested by Student's t test for paired and unpaired data as appropriate. Linear regression lines were calculated by the method of least squares.

Results

Pressor Effect of Angiotensin II

As shown in figure 1, the 11 rats (dextrose-treated) that had angiotensin II infused by osmotic pump for 9 days at 30 ng/min exhibited on the ninth day a baseline MAP of 147 ± 3 mm Hg, i.e., 22 mm Hg higher than that of the sham-pump control animals, which was at 125 ± 2 mm Hg (p < 0.001) and higher than that of normotensive control animals infused acutely with the same dose (128 ± 4 mm Hg, p < 0.01). Tenfold higher doses of angiotensin II, i.e., 300 ng/min infused acutely, induced a range of BP elevation similar to that of the chronically infused group, and this was associated with marked bradycardia of 372 ± 20 bpm as compared to 490 ± 9 bpm in sham controls (p < 0.01). In contrast, heart rates were not different between the sham and chronic angiotensin II groups (490 ± 9 vs 485 ± 14 bpm respectively).

Assessment of Converting Enzyme Inhibition

To estimate the effect of converting enzyme blockade, bradykinin sensitivity was evaluated in all animals. Figure 2 illustrates the approach in one rat. The induced pressure drop was linearly related to the log of the bradykinin dose (mean R = 0.969 ± 0.005; n = 21). Based on this relationship, the dose J/kg for bradykinin was calculated for each rat, i.e., the dose of bradykinin expressed per kg body weight that produced a reduction in MAP of 30 mm Hg.

Figure 3 compares the relative bradykinin sensitivity of the All-infused groups depending on whether they received dextrose or the converting enzyme inhibitor. The group treated by converting enzyme inhibition had a more than tenfold increase in sensitivity to bradykinin, i.e., their dose J/kg was 0.10 ± 0.01 µg/kg as compared to 1.21 ± 0.21 µg/kg in the dextrose-treated group (p < 0.001), thus confirming the effect of converting enzyme blockade on kininase II activity. Plasma angiotensin-converting enzyme activity reflected the blockade as well, being 176 ± 16 nmoles/ml/min in the dextrose-treated group and 32 ± 7 nmoles/ml/min in the rats with converting enzyme inhibition (p < 0.001).

Comparison of Dextrose and CEI During Angiotensin II Infusion

Some baseline and experimental data of the groups of rats that underwent chronic angiotensin II infusion are shown in table 1. There was no difference in age or weight. Baseline MAP on the 9th day of angiotensin II infusion was similar, i.e., 147 ± 3 mm Hg for the
dextrose-treated group and 145 ± 6 mm Hg in the rats with converting enzyme inhibition. Heart rate was similar also, as was the maximal increase in heart rate induced by bradykinin administration and the decrease in body weight observed over the 9 days of angiotensin II infusion. Plasma norepinephrine and epinephrine were similar, and plasma potassium and sodium concentration also did not differ.

Figure 4 depicts the effect of saralasin administration on MAP and heart rate. Endogenous PRA is also shown. The BP levels immediately prior to saralasin infusion were similar for the CEI- and dextrose-treated rats, at 146 ± 7 and 155 ± 5 mm Hg. When the effect of angiotensin II was inhibited by saralasin, both had large reductions in BP, to 102 ± 7 and 111 ± 7 mm Hg respectively. These decreases were not different between groups, being 44 ± 5 and 43 ± 7 mm Hg. Both had a small, but significant, increase in heart rate during angiotensin II blockade, which reversed during recovery from saralasin. Final BP levels also did not differ, at 145 ± 7 and 151 ± 7 mm Hg for the CEI- and dextrose-treated group respectively. Plasma renin activity was markedly suppressed during chronic angiotensin II infusion, at 2.8 ± 0.7 ng/ml/hr (CEI-treated) and 1.5 ± 0.6 ng/ml/hr (dextrose-treated) compared to 18.3 ± 3 ng/ml/hr in the sham-pump group (p < 0.001).

Metabolic Studies in Chronic Angiotensin II-Infused Animals

On the first day following pump placement there was a marked decrease in food intake, from 15.1 ± 0.4 to 8.3 ± 0.72 g (p < 0.001, n = 8) followed by a gradual recovery over 6 days to baseline levels. There was no difference between dextrose- and CEI-treated rats. Supplemental water intake increased beginning on the first day of angiotensin II infusion and became statistically significant from Day 2 on. There was no evident difference between groups receiving dextrose or the converting enzyme inhibitor. Overall supplemental mean daily water intake doubled during pump infusion from 4.12 ± 0.51 to 9.02 ± 1.3 ml (p < 0.01).

Daily weight changes during pump infusion were compared to the baseline weight. For comparison, the same animals receiving dextrose gavage during the week prior to pump placement showed a weight in-

![Figure 2](image-url)  
**Figure 2.** Vasodepressor response to exogenous bradykinin. The upper panel shows the superimposed BP tracings obtained 15 seconds after bolus injection of serial doses of bradykinin. The fall in BP with each dose is plotted in the bottom panel against the bradykinin dose on a logarithmic scale. The resulting line allows calculation of a standard dose to obtain a 30 mm Hg fall, the dose = \( \frac{t}{kg} \), a numerical expression of the sensitivity to bradykinin.

![Figure 3](image-url)  
**Figure 3.** Bradykinin sensitivity and plasma converting enzyme activity in rats receiving chronic angiotensin II infusion. The CEI-treated group requires much less bradykinin for the same BP drop and hence is more sensitive to bradykinin. Plasma converting enzyme activity is greatly reduced in the CEI group, confirming effective enzyme blockade.
crease of 7.4 ± 4.2 g. Sham-pump control animals exhibited a transient weight loss of 10.6 ± 1.4 g immediately after pump placement, but regained weight quickly and by the end of 1 week reached a level comp-

<table>
<thead>
<tr>
<th>Day &amp; Measurement</th>
<th>Dextrose (n = 11)</th>
<th>CEI (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline MAP (mm Hg)</td>
<td>147 ± 3</td>
<td>145 ± 6</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>485 ± 14</td>
<td>482 ± 20</td>
</tr>
<tr>
<td>Maximum Δ heart rate during hypotension with bradykinin (beats/min)</td>
<td>33 ± 9</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>Weight change during chronic AII (g)</td>
<td>-11 ± 3</td>
<td>-13 ± 6</td>
</tr>
<tr>
<td>Plasma sodium (mEq/liter)</td>
<td>140.3 ± 1.2</td>
<td>138.6 ± 1.1</td>
</tr>
<tr>
<td>Plasma potassium (mEq/liter)</td>
<td>4.5 ± 0.14</td>
<td>4.37 ± 0.16</td>
</tr>
<tr>
<td>Plasma norepinephrine (ng/ml)</td>
<td>1.52 ± 0.28</td>
<td>1.15 ± 0.23</td>
</tr>
<tr>
<td>Plasma epinephrine (ng/ml)</td>
<td>1.53 ± 0.35</td>
<td>1.48 ± 0.23</td>
</tr>
</tbody>
</table>

FIGURE 4. Blood pressure and heart rate before and after saralasin infusion. Both groups with active pumps (chronic angiotensin II) have elevated BP (p < 0.001 compared to sham pumps), whether treated with CEI or dextrose. Saralasin infusion confirmed that this elevation is angiotensin-dependent, and the degree of BP fall is similar in both treatment groups. All animals receiving exogenous angiotensin II had effective suppression of endogenous plasma renin activity (p < 0.001, compared to sham pumps).

Discussion
Inhibition of the renin-angiotensin system has emerged as a powerful tool to evaluate the physiologic and pathophysiologic roles of the system. However, drugs used to inhibit angiotensin have properties that limit their value in this regard. All the competitive analogs of angiotensin II, including saralasin, have partial agonist effects, which may vary with the state of sodium balance. Using these antagonists, one may therefore underestimate the participation of the renin system. Angiotensin-converting enzyme inhibiting agents tend to produce greater BP drops than
Fig. 5. Fractional urinary sodium and potassium excretion during chronic angiotensin II infusion. Sodium excretion was quite variable after beginning angiotensin II, but showed a trend toward increased urinary excretion. Potassium shows an increased excretion that reapproaches baseline at 5 days. No differences were apparent between CEI- and dextrose-treated animals, with the exception of the first day of CEI, when urinary sodium excretion was less in the CEI rats (p < 0.005 when compared to dextrose).

Saralasin, particularly when renin levels appear "normal" or "low,"20 Interpretation of these results as reflecting exclusively the effect of angiotensin II removal has been challenged, since the degree to which long-term blockade of converting enzyme is influenced by nonangiotensin mechanisms is unknown.

It is particularly uncertain how much bradykinin accumulation or potentiation contributes to the CEI effects. Indeed, reports of short-term clinical studies have described elevation of plasma bradykinin levels following inhibition of converting enzyme with intravenous teprotide, and lower BP than apparently could be accounted for by diminished angiotensin II levels.12, 21 Other investigators, however, have found neither an increase in bradykinin22 nor a hypotensive effect of CEI when angiotensin II was administered concomitantly.23 Moreover, chronic administration of captopril has not resulted in any long-term increase in circulating bradykinin,24 which may not be surprising in light of the other known route of bradykinin degradation. Even without finding increased venous levels, researchers may argue nonetheless that following CEI a greater quantity of bradykinin reaches the peripheral arterioles in active form, thereby affecting BP and regional blood flow, because it escapes part of its normal removal during the first passage through the lung. Whether there is local bradykinin generation or action in specific vascular beds is not known. Hence, nearly every report describing the use of converting enzyme inhibitors has qualified its discussion...
of the findings based on the possibility of multiple modes of action of these drugs.\textsuperscript{14-21}

There is a clear need for data concerning the longer term effects of CEI without lowered angiotensin II levels. Recently, some investigators described the effect of long-term converting enzyme blockade in salt-depleted dogs. Association of an angiotensin II infusion clearly reversed the observed BP reduction, which was not the case when aldosterone was infused.\textsuperscript{26} However, since there was no control group receiving angiotensin II alone, that study was not designed to rule out a possible angiotensin-independent hypertensive effect of converting enzyme blockade. In the present experiments, the effects of CEI were dissociated from lowering angiotensin II levels by providing continuously exogenous angiotensin II.

Chronic angiotensin II infusion has been shown to produce hypertension in animals and humans. With the recent development of miniaturized osmotically driven pumps, this has become technically feasible in rats. With this technique, sustained BP elevation was produced with doses that raised BP much less when given acutely. This confirms earlier findings in other species.\textsuperscript{20-22} Increased sensitivity to angiotensin II given chronically has been attributed to a variety of factors, including sodium retention and altered receptor responsiveness to angiotensin II.\textsuperscript{24} Since in the present study angiotensin II was infused at a relatively high rate, which was clearly pressor, no sodium retention occurred and thus the first argument cannot explain the observed increased sensitivity to angiotensin II. Normal heart rate and normal response to alterations in the BP in spite of elevated systemic pressures, i.e., "resetting of baroceptors," were invariably observed, but this is unlikely to account for the BP elevation, as has been commented upon by others.\textsuperscript{25, 26}

To evaluate the efficacy of converting enzyme inhibition, the sensitivity to exogenous bradykinin and plasma angiotensin-converting enzyme activity were measured. Angiotensin I blockade was not tested, since the animals made hypertensive by angiotensin II infusion were expected to have a decreased sensitivity to angiotensin I, rendering results difficult to interpret. The dosage of captopril was deliberately large, known to reach maximal effect rapidly and to block the pressor effect of angiotensin I in conscious rats for at least 12 hours.\textsuperscript{27} As expected, the CEI-treated group showed a more than tenfold increase in vasodepressor sensitivity to bradykinin. Plasma values of converting enzyme activity were markedly diminished. Together, these results established functional converting enzyme blockade.

In spite of marked blockade, the results of the present study are most striking for the lack of any sustained hemodynamic effect of chronic CEI in the presence of angiotensin II. Accordingly, there is no evidence of a direct vasodepressor effect, either in the form of a difference between the dextrose- and CEI-treated groups in baseline BP or in the magnitude of the BP fall after removal of the pressor effect of circulating angiotensin II by the infusion of saralasin. Heart rates were comparable, as was the heart rate response to bradykinin-induced hypotension. Moreover, there was no evidence of renin stimulation by converting enzyme inhibition per se. One well-recognized finding during converting enzyme blockade is a rise in PRA.\textsuperscript{16, 27} This has been attributed to the interruption of a negative feedback loop in which angiotensin II suppresses renin release, although intrarenal vasodilator mechanisms via bradykinin and/or prostaglandins have also been considered.\textsuperscript{18} In all animals receiving angiotensin II infusion chronically, regardless of whether treated with dextrose or CEI, endogenous PRA was well below levels obtained in normotensive conscious animals under similar sampling conditions (fig. 4). This supports the hypothesis that the angiotensin II feedback is indeed the predominant, if not the only, renin suppressing force.

The results obtained during the metabolic studies are in agreement with the BP and heart rate findings on the final day. All animals receiving angiotensin II demonstrated an increase in supplemental water drinking, a transient decrease in food intake, and an ongoing weight loss not attributable to the placement or presence of the pump alone. Daily urinary fractional excretion of potassium showed a prompt and sustained increase over the first 5 days in both dextrose and CEI groups. Urinary sodium excretion was more variable and almost certainly influenced by multiple forces. It was not the objective of this study to elaborate on the mechanisms of angiotensin II influencing sodium or potassium metabolisms, a subject that has been extensively investigated and reviewed.\textsuperscript{7}

Most relevant is the finding that there was little difference between groups receiving dextrose or CEI, in spite of greatly enhanced bradykinin sensitivity in the CEI-treated group. During the first day of medication, i.e., the third day of angiotensin II administration, there was a uniform decrease in urinary sodium excretion in the CEI group. On first view, this might be taken as evidence against a bradykinin effect, since the action of bradykinin on the kidney is primarily natriuretic.\textsuperscript{36, 37} However, one could argue that the most powerful effect may have been a hypotensive one, potent enough to counter the angiotensin-induced systemic pressure increase and delay pressure diuresis, and perhaps to allow a mineralocorticoid-induced sodium retention to go unopposed. This point cannot be resolved with the data at hand as pressures were not measured systematically that day, but in any event, from the next day forward there was no apparent difference between the treatment groups. The apparent sodium retention observed on the first day of CEI treatment, which did not persist, is consistent with the recent findings of Carretero and Scicli\textsuperscript{28} that anti-bradykinin antibodies may modify the pressure response immediately following CEI but have no effect during chronic treatment.

Taken together, the results of these experiments suggest that, in the presence of angiotensin II, long-term CEI has no apparent effect. This is evidence against a significant mechanism of CEI independent of angiotensin-blockade.
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Converting enzyme inhibition during chronic angiotensin II infusion in rats. Evidence against a nonangiotensin mechanism.

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doi: 10.1161/01.HYP.3.2.269

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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