Converting Enzyme Inhibition and Renal Tissue Angiotensin II in the Rat

Donald R. Allan, John A. McKnight, Imre Kitor, Caroline M. Coletti, Norman K. Hollenberg

Abstract  Multiple observations suggest local control of renal function via an intrarenal renin-angiotensin system, including evidence for local angiotensin (Ang II) production. Our first goal was to examine renal tissue Ang I:Ang II relations to ascertain whether Ang II formation differs in the circulation and in renal tissue. We have recently shown an authentic Ang II/Ang I ratio of 1.5:1 in renal lymph, the opposite of the Ang II:Ang I ratio in plasma. Our second goal was to examine the influence of maximal converting enzyme inhibition on these relations in plasma and in renal tissue. We used two converting enzyme inhibitors with differing solubility, on the premise that tissue penetration and action might differ on that basis. We measured Ang I and Ang II in plasma and renal tissue of rats given an intravenous dose of either vehicle, enalapril, or ramipril, over a wide dose range, from 0.1 to 10.0 mg/kg IV. Renal and plasma angiotensin concentrations were measured by high-performance liquid chromatography and radioimmunoassay. Whereas the Ang I concentration in normal rat plasma (273±84 fmol/mL) was over threefold the plasma Ang II concentration (83±12 fmol/mL), the ratio was reversed in the kidney (Ang II, 178±12 versus Ang I, 91±3 fmol/g; P<.001). Although ramipril and enalapril induced an indistinguishable dose-related acute fall in blood pressure and plasma Ang II concentration, lower enalapril doses were less effective in reducing renal tissue Ang I:Ang II conversion and Ang II concentration (P<.025). The results confirm differences in the intrarenal and plasma compartments for Ang I and Ang II, compatible with different patterns of Ang II generation and suggest that converting enzyme inhibitors differ in their ability to reach and act in a large intrarenal compartment, but their maximal influence leaves substantial authentic Ang II generation. (Hypertension. 1994;24:516-522.)

Key Words  • ramipril  • enalapril  • renin

A long-standing and widely held opinion that the renin-angiotensin system functions primarily as an endocrine system has been challenged, and there is growing interest in the contribution of local tissue renin systems to local angiotensin II (Ang II) formation, an autocrine or paracrine function. Several lines of evidence have favored a contribution of a local renin-angiotensin system in the kidney. These include the crucial location of the available angiotensin-converting enzyme (ACE) at the sites of renin formation and of the vascular action of angiotensin, Ang II concentrations in the renal tissue fluids and lymph draining the kidney that are far too high to be accounted for on the basis of delivery by way of the arterial tree, and multiple additional observations that favor a major element of local production and action.

Another approach to assessing the state of tissue renin-angiotensin systems has emerged with improvements in the methods for separation and measurement of angiotensin peptide and fragment concentration in tissues. One goal of the present study was to ascertain whether the ratio of authentic Ang I to Ang II in renal tissue resembled that in renal lymph, where Ang II concentration exceeds that of Ang I, or in plasma, where Ang I is found in excess. Second, although two studies have documented the ability of ACE inhibitors to influence Ang II concentration in the kidney, only a limited dose range was assessed, so it is not clear whether maximal blockade was achieved. Our second goal was to define the relation between two ACE inhibitors, ramipril and enalapril, and their influence on plasma and renal tissue Ang II concentrations. We used two different ACE inhibitors for two reasons: the first involved the generalizability of the observations; the second involved the possibility that differences in their ability to block tissue systems might be uncovered. Because of the confounding effects of angiotensin fragments on the measurement of immunoreactive Ang II, all assays were performed on HPLC-separated specimens for measurement of authentic plasma and tissue Ang I and Ang II concentrations.

Methods

Thirty-seven male Sprague-Dawley rats weighing 250 to 300 g were obtained from Charles River Laboratories. They were housed and cared for according to the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHHS publication No. [NIH] 85-23, revised 1985). Rats were fasted for 18 hours before surgery but allowed free access to water.

On the day of the experiment, rats were anaesthetized with sodium pentobarbital (75 mg/kg IP) (Anthony Products Co) with a maintenance infusion of 0.20 mg/kg per minute for the duration of the experiment. The trachea was cannulated with PE-205 tubing and the rat allowed to breathe spontaneously. A carotid catheter (PE-50) was connected to a Statham pressure transducer (model P23XL) and Grass polygraph (model 7) for...
monitoring of arterial blood pressure and heart rate. A jugular vein was cannulated with a triaxial catheter constructed by placing three PE-10 tubes in a straight pediatric angiography catheter, sealed to prevent back leak, for the intravenous administration of the agents used. After a 30-minute stabilization period, 0.30 mL of either vehicle (5% dextrose in water [D,W], n=5), enalapril (0.1 [n=5], 1.0 [n=6], or 10.0 [n=4] mg/kg dissolved in D,W), or ramipril (0.1 [n=6], 1.0 [n=6], or 10.0 [n=5] mg/kg dissolved in D,W) was administrated over 5 minutes. The doses were chosen on the basis of a pilot study that revealed activity in the indexes of interest at the doses used and no response to a dose 10-fold lower (0.01 mg/kg). Thirty minutes later, an abdominal incision was made, both renal arteries were clamped, and the right kidney was removed within 20 seconds. The excised kidney was handed to a second worker for immediate homogenization. Approximately 3 mL of blood was drawn through the carotid artery line into tubes containing EDTA and immediately centrifuged at 2600 rpm in a Du Pont centrifuge at 4°C (New England Nuclear). The plasma was aspirated, transferred to a polypropylene tube containing 2 mL of 8 mol/L urea, and immediately frozen and stored at −70°C. The rat was then killed with intravenous pentobarbital.

Tissue Processing

Fresh tissue was homogenized immediately. After being weighed on a rapid electronic balance, the right kidney was blended once in 4 mL of 8 mol/L urea containing 0.1% Triton X-100 and a high-speed tissue homogenizer for 1 to 2 minutes (Biohomogenizer, Biospec Products). More complete homogenization was then accomplished in a 55-mL ice-cooled, motor-driven borosilicate glass vessel fitted with a serrated polytetrafluoroethylene pestle (Potter-Elvehjem homogenizer, Thomas Scientific). Urea, a chaotropic agent, disrupts enzyme activity and inactivates angiotensinases involved in angiotensin production and degradation. This mixture was centrifuged at 4°C at 3000 rpm in a tabletop centrifuge. The supernatant was collected and divided equally into polyethylene test tubes containing 50 μL of 10% glycerol and placed in a SpeedVac concentrator overnight. The dried samples containing the angiotensin peptides dissolved in the 50 μL glycerol were then ready for solid-phase extraction.

Sep-Pak C18 cartridges (3.0 mL, 500 mg) (Waters Associates) were loaded into a vacuum extractor system (Vac Elut SPS 24, Analytech International) and activated by washing with 2.0 mL of 0.1% triethanolamine (TEA), followed by 2.0 mL of 80% methanol with 0.1% TEA and an additional 4.0 mL of 0.1% TEA. The dried tissue sample was reconstituted in 4 mL saline, passed through the Sep-Pak C18 cartridge at 1.0 mL/min, and subsequently rinsed with 1.0 mL saline. Peptide elution was accomplished with 4.0 mL of 80% methanol. The collection tubes containing 50 μL of 10% glycerol were placed in a SpeedVac drier overnight. Plasma samples were thawed and extracted in the same manner in preparation for high-performance liquid chromatography (HPLC). For assessment of the recovery of the extraction methodology, five samples of previously HPLC-purified tritiated Ang II were extracted on Sep-Pak columns and processed in an identical manner. The eluates were mixed with 4 mL scintillation fluid and counted. Recovery of tritiated Ang II was 75±5% using this extraction technique. Results presented are not corrected for these losses.

Peptide Measurement

Our HPLC and radioimmunoassay methodology for the measurement of angiotensin peptides in tissue other than kidney has been reported.24-25 Briefly, the dried samples were reconstituted in 550 μL of sample solvent (10 mmol/L sodium acetate, 10 mmol/L TEA, 5% methanol, 0.15 mmol/L NaH2PO4, 10 mL assay buffer per 500 mL) and injected into a 3-μm C18 15 cm×4 mm column (Merck Sharp & Dohme). HPLC equipment consisted of an LKB 2150 pump with a dynamic mixer, a 2152 LKB controller, and a 2211 LKB fraction collector. Solution A contained 10 mmol/L TEA and 10 mmol/L sodium acetate adjusted to a pH of 6.2 before mixing with methanol to a final concentration of 30%. Solution B was prepared similarly except that it contained 80% methanol. The following gradient was used: 0 minutes, 0% B; 5 minutes, 14% B; 30 minutes, 14% B; 35 minutes, 20% B; 50 minutes, 40% B; 65 minutes, 54% B; and 80 minutes, 54% B. The flow rate was 0.55 mL/min. One-minute fractions were collected in test tubes containing 50 μL of 10% glycerol and 150 μL of 50% assay buffer and were subsequently dried overnight. Elution times and their standard deviations of the angiotensin peptides were determined by repeated injections of 4-nmol aliquots of the synthetic peptides into the HPLC apparatus and recording the absorbance with a spectrophotometer tuned to 214 nm and connected to a computer loaded with the CHROMATOCHART software package (R. Maciel Inc). The Ang II elution time was checked periodically with tritiated Ang II and also by observing the visible elution time of phenol red, which was consistently 2 minutes ahead of the Ang II with this gradient. With the use of this gradient the elution times of the various peptides tested—peptide 4-8, Ang II, peptide 1-9, peptide 3-8, Ang III, Ang I, and des-Asp Ang I—were consistent and the peptides well separated (Fig 2). HPLC recovery was analyzed using five duplicate HPLC-purified, tritiated Ang II samples. Eluates contained 94±3% of the β-activity seen in
their duplicate counterparts that were not subjected to HPLC.

Results are not corrected for these losses.

Radioimmunoassay was performed on the dried HPLC eluates. Standards were prepared the day before by serial dilutions of premixed aliquots of Ang I and Ang II (Peninsula Laboratories) mixed in polyethylene tubes containing 50 \( \mu \)L glycerol and dried in a SpeedVac concentrator overnight. The Ang II radioimmunoassay was performed on fractions 27 through 62 by addition to the tubes of 2500 cpm of \( ^{125}\text{I}-\text{Ang II} \) tracer (Du Pont–New England Nuclear) dissolved in 50 \( \mu \)L of a 50% buffer solution (0.05 mol/L K_2HPO_4, 0.003 mol/L EDTA, 0.02% sodium azide, 0.01% Triton X-100, and 2.5 mg/mL radioimmunoassay-grade bovine serum albumin) and 100 \( \mu \)L of the 50% buffer solution containing rabbit anti-Ang II antibody (Arnel). The concentration of the anti-Ang II antibody was carefully adjusted to yield a 35% to 40% specific binding after incubation with the tracer solution for 48 hours. This antibody exhibited 100% cross-reactivity with Ang II and Ang III but only 0.1% cross-reactivity with Ang I. Similarly, 50 \( \mu \)L of the 50% buffer solution containing 2500 counts of \( ^{125}\text{I}-\text{Ang I} \) was mixed with 100 \( \mu \)L of solution containing rabbit anti-Ang I antibody in tubes containing fractions 63 through 80, which contain the Ang I and des-Asp Ang I. The anti-Ang I antibody had 100% cross-reactivity with the des-Asp peptide. The samples were incubated for 48 hours at 4°C. Donkey anti-rabbit IgG magnetic separation reagent (200 \( \mu \)L) was added. Fifteen minutes later the samples were placed on magnetic test tube holders (Amersham International). After 10 minutes the supernatants were decanted. The pellet was washed with 750 \( \mu \)L of solution containing 0.1% gelatin, 0.01% Triton X-100, 0.05 mol/L NaCl, 0.10 mol/L MgC, and 0.02% sodium azide. Ten minutes later the tubes were decanted again.

The radioactivity of the pellets was recorded for 3 minutes with a Micromedic 4/200 gamma counter coupled to an IBM AT computer loaded with RIA-AID software (R. Maciel Inc). Counts were converted to angiotensin concentration values using this software. With this method, the limit of detection (B_0±2 SD) was 0.2 fmol per tube. The sensitivity for detection of Ang I and Ang II in plasma was 0.7 fmol/mL. The nonspecific binding was routinely less than 1%. The PEAKFIT software package (Jandel Scientific) was used to analyze the area under each peak for calculation of the total angiotensin peptide per sample.

Statistical Analysis

Mean values are presented with the SEM as the index of dispersion. The index of angiotensin conversion was calculated as Ang II/(Ang I+Ang II) to follow the mass action equation. ANOVA was used to assess each dose-response relation for blood pressure and plasma and tissue Ang II concentrations. Dunnett’s \( t \) test, a form of ANOVA, was used for comparisons with baseline blood pressure. Student’s \( t \) test was then used to ascertain whether statistically significant differences occurred at any dose. Nonparametric analysis with the Wilcoxon rank sum test was used to assess responses when a normal distribution of the data was doubtful. The null hypothesis was rejected at a value of \( P<.05 \).

Chemicals

Enalapril was kindly provided by Merck Sharp & Dohme and ramipril by Hoechst Pharmaceuticals. Methanol Omnisolv was obtained from EM Science. Potassium phosphate, gelatin, EDTA, Triton X-100, and magnesium chloride were purchased from Serva Biochemicals. Sodium azide, bovine serum albumin, sodium acetate, urea, and sodium chloride were purchased from Sigma Chemical Co.

Results

Six rats treated with vehicle remained stable throughout the experimental procedure without any change in their mean blood pressures, whereas treatment with ramipril or enalapril lowered blood pressure significantly (Table 1). Thirty minutes after the intravenous bolus of the 0.1 mg/kg dose of either ACE inhibitor, mean blood pressure remained 14 mm Hg below the pretreatment baseline \( (P<.01) \). The 1.0 mg/kg dose of either ACE inhibitor lowered mean arterial blood pressure further, by 20 mm Hg \( (P<.01) \). The highest dose of ACE inhibitor, 10.0 mg/kg, resulted in a small but unambiguous further decline in blood pressure, both at the nadir and at 30 minutes \( (P<.01) \). The two drugs were equipotent in their ability to lower blood pressure,

![Fig. 2. Plot shows elution profile of angiotensin (Ang) peptides after high-performance liquid chromatographic separation with the gradient used in this study. Al indicates angiotensin I.](image)

**Table 1. Blood Pressure Response to Enalapril and Ramipril**

<table>
<thead>
<tr>
<th></th>
<th>Baseline, mm Hg</th>
<th>Maximal Effect, mm Hg</th>
<th>30 Minutes After Dose, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle</strong></td>
<td>112±10</td>
<td>109±10</td>
<td>110±10</td>
</tr>
<tr>
<td><strong>Ramipril</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>123±8</td>
<td>93±10</td>
<td>108±8</td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>118±7</td>
<td>88±6</td>
<td>99±5</td>
</tr>
<tr>
<td>10.0 mg/kg</td>
<td>106±3</td>
<td>73±10</td>
<td>93±4</td>
</tr>
<tr>
<td><strong>Enalapril</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>117±4</td>
<td>93±7</td>
<td>103±5</td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>127±5</td>
<td>89±5</td>
<td>106±2</td>
</tr>
<tr>
<td>10.0 mg/kg</td>
<td>112±3</td>
<td>75±8</td>
<td>95±6</td>
</tr>
</tbody>
</table>
Angiotensin and the Kidney

Allan et al

I

RUUPRIL

I

ENALAPRIL

ACE INHIBITOR Dose (mg/kg)

FK3

3. Line graph shows plasma angiotensin II (Ang II) concentration in vehicle-treated rats and rats treated with graded doses of the angiotensin-converting enzyme (ACE) inhibitors enalapril and ramipril. Fall in plasma Ang II concentration was identical with the two agents; the lowest dose used was near the top of the dose-response relation.

with no significant difference in the observed fall in blood pressure at any dose or time (Table 1).

Plasma Ang II concentration in the vehicle-treated rats was 83.2±12.1 fmol/mL (Fig 3) and fell significantly (P<.001) to an essentially identical level with the lowest dose (0.1 mg/kg) of enalapril (33.0±1.7 fmol/mL) and ramipril (31.3±3.6 fmol/mL). Increasing the ACE inhibitor dose 100-fold induced a small further decrement (P<.01; Fig 3 and Table 2), but there was no significant difference in the ACE inhibitor-induced fall in plasma Ang II concentration at any dose of ramipril or enalapril.

The Ang II concentration in renal tissue of vehicle-treated rats was 178±12 fmol/g (Fig 4), substantially higher than the plasma concentration. Both ACE inhibitors induced a dose-related fall in renal tissue Ang II concentration, with an essentially identical response to the maximal dose (10 mg/kg) of enalapril (60±12 fmol/g) and ramipril (56±6 fmol/g) used in this study (Fig 4 and Table 2). At lower ACE inhibitor doses, however, a different response pattern emerged. With the lowest ACE inhibitor dose used (0.1 mg/kg), enalapril induced no change in renal tissue Ang II concentration (178±24 fmol/g), whereas ramipril induced a large fall (104±17 fmol/g, P<.025). For the entire dose-tissue response, the two agents differed (P<.025) despite convergence at the highest doses.

In vehicle-treated rats the plasma Ang I concentration (273±84 fmol/mL), as anticipated, was substantially higher than the plasma Ang II concentration, so that the ratio was more than 3:1 (3.3±1.9, Table 2). Conversely, in renal tissue the concentration of Ang I (90.8±3.4 fmol/g) was lower than that of Ang II (178±12 fmol/g), so that the ratio was reversed (0.51±0.05, P=.026). For the entire dose-tissue response, the two agents differed (P<.001) despite convergence at the highest doses.

The identical enalapril dose, 0.1 mg/kg, failed to alter renal tissue Ang II, but renal tissue Ang I concentration was increased

Table 2. Renal Tissue and Plasma Ang I, Ang II, and Conversion Index

<table>
<thead>
<tr>
<th>Group</th>
<th>Ang I, fmoI/g</th>
<th>Ang II, fmoI/g</th>
<th>Ratio</th>
<th>Conversion Index</th>
<th>Ang I, fmoI/g</th>
<th>Ang II, fmoI/g</th>
<th>Ratio</th>
<th>Conversion Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>91±3.4</td>
<td>178±12</td>
<td>0.51±0.05</td>
<td>0.66±0.012</td>
<td>273±84</td>
<td>83±20.6</td>
<td>3.3±1.9</td>
<td>0.30±0.077</td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>Enalapril</td>
<td>231±56t</td>
<td>178±24</td>
<td>1.3±0.7</td>
<td>0.47±0.090t</td>
<td>865±293*</td>
<td>33±1.7t</td>
<td>26.2±11.5†</td>
</tr>
<tr>
<td></td>
<td>Ramipril</td>
<td>291±88t</td>
<td>104±17t†</td>
<td>2.8±1.1*</td>
<td>0.33±0.087t††</td>
<td>742±237*</td>
<td>31±3.6t</td>
<td>23.9±3.4†</td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>Enalapril</td>
<td>260±97†</td>
<td>90±7.5†</td>
<td>2.9±0.9t</td>
<td>0.33±0.074†</td>
<td>898±109*</td>
<td>31±7.4†</td>
<td>29.0±9.8†</td>
</tr>
<tr>
<td></td>
<td>Ramipril</td>
<td>178±48†</td>
<td>70±11†</td>
<td>2.5±1.7†</td>
<td>0.33±0.060†</td>
<td>427±145*</td>
<td>23±3.1†</td>
<td>18.9±3.1†</td>
</tr>
<tr>
<td>10.0 mg/kg</td>
<td>Enalapril</td>
<td>257±81t</td>
<td>60±11.6†</td>
<td>4.3±2.7†</td>
<td>0.26±0.090t</td>
<td>716±154t</td>
<td>21±3.7†</td>
<td>34.1±7.1†</td>
</tr>
<tr>
<td></td>
<td>Ramipril</td>
<td>243±52†</td>
<td>56±6.0†</td>
<td>4.3±0.8†</td>
<td>0.20±0.038†</td>
<td>566±91t</td>
<td>28±2.9†</td>
<td>20.2±3.1†</td>
</tr>
</tbody>
</table>

Ang indicates angiotensin. Ang II conversion index was calculated as Ang II/(Ang I+Ang II); ratio is Ang I to Ang II.

*P<.05, †P<.01 compared with vehicle control.

‡P<.05, enalapril vs ramipril.
we have made in lymph. 26 Although ACE inhibition was undeniably effective in reducing both plasma and renal tissue, confirming and extending a recent observation substantially higher in plasma, the ratio was reversed in renal also made. Although Ang I concentration was substantially higher Ang II concentrations in renal tissue, on the other hand, conversion was more limited to 30 minutes in this study. In the study by Fox et al,18 the exposure to enalaprilat by bolus followed by infusion in a dose near the midpoint of the range used duration of exposure to the ACE inhibitor, which was limited to 30 minutes in this study. In the study by Fox et al,18 the exposure to enalaprilat by bolus followed by infusion in a dose near the midpoint of the range used during the interval when the methods used in this study were being developed, Fox et al18 reported a similar experience with the trade-offs that exist. Although it would be preferable to use unanesthetized animals, harvesting the kidneys after a guillotine procedure followed by rapid freezing of the tissue for later processing, in agreement with Fox et al,18 we found that even apparently minor variation in the time required to harvest and freeze the tissues led to an unacceptable level of variability. For that reason, we adopted an approach similar to theirs. Rapid tissue handling is necessary to prevent massive Ang II production and degradation. To reduce further this problem, we used 8 mol/L urea as a chaotropic agent to limit angiotensin metabolism.24,25 Not only did anesthesia and surgery to harvest the kidneys activate the renin system, the studies were performed after an overnight fast, a maneuver that induces a state of sodium balance in which renal excretion of sodium falls to very low levels and the renin system is activated.28,29 The plasma and renal tissue Ang I and Ang II concentrations reported in the present study represent an activated system.

Over the 100-fold dose range of the two ACE inhibitors used, both blood pressure and plasma Ang II concentration showed an essentially identical response. The lowest dose, 0.1 mg/kg, induced a near nadir in both plasma Ang II concentration and blood pressure. Increasing the dose 100-fold produced only a small additional parallel response of both blood pressure and plasma Ang II. The top dose used, 10 mg/kg, is the highest ramipril dose that can be given in a convenient volume. The findings suggest that the acute depressor response to the ACE inhibitors reflected primarily the fall in plasma Ang II level and not their action in tissues.

At the highest ACE inhibitor dose used, plasma and especially tissue Ang II levels remained well above threshold sensitivity. Complete blockade of Ang II formation clearly had not been achieved. Others have described a fall in plasma Ang II levels to below the threshold of detection with ACE inhibition.30 Whether the difference reflects the conditions of our experiment, in which the renin-angiotensin system was activated, or technical differences in measurement cannot be determined. Another potentially relevant variable is the duration of exposure to the ACE inhibitor, which was limited to 30 minutes in this study. In the study by Fox et al,18 the exposure to enalaprilat by bolus followed by infusion in a dose near the midpoint of the range used in this study resulted in a 78% reduction in plasma Ang II levels, a 75% decrease in renal Ang II concentration,

**Discussion**

Recent advances have made the measurement of authentic Ang I and Ang II with reasonable fidelity possible not only in plasma and other body fluids but also in tissue. These techniques allowed us to confirm in this study a number of observations, including the substantially higher Ang II concentrations in renal tissue than in plasma and the ability of ACE inhibitors to reduce not only plasma but also tissue Ang II concentration. A number of novel observations were also made. Although Ang I concentration was substantially higher in plasma, the ratio was reversed in renal tissue, confirming and extending a recent observation we have made in lymph.26 Although ACE inhibition was undeniably effective in reducing both plasma and renal tissue Ang II concentrations, both ACE inhibitors used were substantially less effective in the tissue than in the vascular compartment until very large doses were used. The two ACE inhibitors induced an identical maximal reduction in plasma and renal tissue Ang II concentrations. At maximal response, substantial residual Ang II was present. Moreover, despite a similar relation between dose and influence on plasma Ang II level, the two ACE inhibitors used demonstrated very different dose-response relations in the renal tissue. Each of these observations merits discussion, but only after consideration of technical factors.

Measurement of renal tissue Ang II concentration reflects recent technical innovation, and broad agreement has not yet been reached on the ideal approach. During the interval when the methods used in this study were being developed, Fox et al18 reported a similar experience with the trade-offs that exist. Although it would be preferable to use unanesthetized animals, harvesting the kidneys after a guillotine procedure followed by rapid freezing of the tissue for later processing, in agreement with Fox et al,18 we found that even apparently minor variation in the time required to harvest and freeze the tissues led to an unacceptable level of variability. For that reason, we adopted an approach similar to theirs. Rapid tissue handling is necessary to prevent massive Ang II production and degradation. To reduce further this problem, we used 8 mol/L urea as a chaotropic agent to limit angiotensin metabolism.24,25 Not only did anesthesia and surgery to harvest the kidneys activate the renin system, the studies were performed after an overnight fast, a maneuver that induces a state of sodium balance in which renal excretion of sodium falls to very low levels and the renin system is activated.28,29 The plasma and renal tissue Ang I and Ang II concentrations reported in the present study represent an activated system.

Over the 100-fold dose range of the two ACE inhibitors used, both blood pressure and plasma Ang II concentration showed an essentially identical response. The lowest dose, 0.1 mg/kg, induced a near nadir in both plasma Ang II concentration and blood pressure. Increasing the dose 100-fold produced only a small additional parallel response of both blood pressure and plasma Ang II. The top dose used, 10 mg/kg, is the highest ramipril dose that can be given in a convenient volume. The findings suggest that the acute depressor response to the ACE inhibitors reflected primarily the fall in plasma Ang II level and not their action in tissues.

At the highest ACE inhibitor dose used, plasma and especially tissue Ang II levels remained well above threshold sensitivity. Complete blockade of Ang II formation clearly had not been achieved. Others have described a fall in plasma Ang II levels to below the threshold of detection with ACE inhibition.30 Whether the difference reflects the conditions of our experiment, in which the renin-angiotensin system was activated, or technical differences in measurement cannot be determined. Another potentially relevant variable is the duration of exposure to the ACE inhibitor, which was limited to 30 minutes in this study. In the study by Fox et al,18 the exposure to enalaprilat by bolus followed by infusion in a dose near the midpoint of the range used in this study resulted in a 78% reduction in plasma Ang II levels, a 75% decrease in renal Ang II concentration,
and approximately a 2.8-fold increase in renal Ang I content in 1 hour. In the study by Campbell et al., rats were exposed to the ACE inhibitor perindopril for 7 days. At the end of this period of exposure, intrarenal Ang II concentration fell to approximately 14% of control. Thus, the available data suggest that the duration of exposure is not the crucial variable limiting the fall in plasma and tissue Ang II levels after ACE inhibition. Campbell et al. reported that tissue Ang I levels decreased more rapidly with prolonged ACE inhibition, whereas we found a sharp rise even with the lowest ACE inhibitor doses used in this short-term study, as anticipated. Prolonged ACE inhibition and reduced Ang II formation could have led to a reduction in angiotensinogen production in the long-term study and thus a fall in Ang I.

One hypothesis that we tested was that two ACE inhibitors might differ in their capacity to inhibit the tissue system, reflecting different chemical and functional characteristics. To test this hypothesis with agents that must differ at least somewhat in potency, we used a wide range of ACE inhibitor doses to identify a range that induces an identical reduction in circulating Ang II concentration and the maximal response. With the lowest dose of the ACE inhibitors used, 0.1 mg/kg, there was an identical fall in plasma Ang II concentration, to near nadir levels, and yet a strikingly different fall in the tissue Ang II level. With increasing doses, as anticipated, that difference disappeared. In agreement with these observations, Richler et al. compared the influence of ramipril and enalapril on renal blood flow in rats after 8 days of treatment; despite an equivalent blood pressure fall, ramipril was more effective at increasing renal blood flow. That observation suggests a functional parallel to the differences in tissue Ang II concentration in the present study and that the difference might be sustained over time.

ACE inhibitors have a wide range of physical and chemical characteristics that could lead to differences in their ability to act in tissues. Among the possible determinants of tissue ACE inhibitor activity, prodrug activation by kidney esterases, variation in tissue penetration based on lipophilicity, differing affinity of the agents for the ACE enzyme, and tightness of binding are all attractive candidates. We chose ramipril and enalapril for this study in part because of their similarities (both are prodrugs with a rather small difference in their potency) and because of their differences. Ramipril is more lipophilic at physiological pH and tends to show tighter binding. Whether these features account for the difference in their action at the tissue level cannot be ascertained from this study.

Most ex vivo studies using either fluorometric spectrophotometry or autoradiography have demonstrated complete inhibition of tissue ACE activity by a variety of ACE inhibitors at the high end of their dose range. Direct measurement of tissue angiotensin provides an alternative estimate of their effectiveness in vivo. Indeed, the relation between Ang I and Ang II might be the most accurate approach to assessing ACE activity. Even at the highest dose of ACE inhibitors, tissue Ang II formation was not abolished. Several possibilities exist to explain this discrepancy with the ex vivo findings. Tissue homogenization when chemical assay is used for the in vitro ACE study may expose the ACE inhibitor in tubular fluid, destined for excretion, to an active site not reached in vivo. In the case of radioautographic localization, ACE in compartments that cannot be reached easily by way of blood vessels might not participate, although it is capable of generating Ang II. Moreover, many of the ex vivo findings rely on the Hip-His-Leu peptide cleavage by ACE. Although the K_m values are of the same magnitude, they are not identical. Pathways that bypass ACE in the formation of Ang II have been described. In Ang II-producing cells, for example, the angiotensin-producing pathway could be separated from other pathways containing enzymes capable of converting Ang I to Ang II or generating Ang II directly. Yet pathways normally separated may intermingle during homogenization. The problem may be complicated further by the time required for ACE assay, which could lead to dissociation of the inhibitor and new enzyme synthesis. Preformed Ang II in storage granules would not be affected. A more prolonged period of ACE inhibition could resolve this issue, although the study by Campbell et al. does not suggest that prolonged exposure to an ACE inhibitor will, even at high doses, abolish Ang II formation. Finally, technical difficulties remain in the radioimmunoassay, in which it is more difficult to resolve signal and noise as the Ang II levels approach zero.

Our finding of the distinctly different ratio of Ang I to Ang II in tissue and plasma in this study agrees with published data and our earlier finding of a high ratio of Ang I to Ang II in renal lymph and superfusates. In all of these studies, authentic Ang I and Ang II were measured by an identical HPLC separation followed by radioimmunoassay. An identical pattern in renal tissue and renal lymph suggests that lymph reflects the tissue compartment more than it does events in plasma. Probably related is the finding of a very high renal tissue Ang II level in this study, which in the light of findings from metabolic clearance studies suggests that local intra-renal Ang II production is very high. The precise location of the compartment in which the Ang II generation occurred was not determined, but immunocytochemical observations suggest that much of the generation occurs in the juxtaglomerular region. The possibility of intracellular generation of Ang II merus consideration. The excessive Ang II relative to Ang I in renal tissue relative to plasma might reflect the storage of the octapeptide in an intracellular compartment. Should this be the case, the assumption implicit in the calculation of conversion rates, that Ang I and Ang II communicate freely, would have been violated. At the moment, the compartments invoked in this discussion are a heuristic device; an anatomic equivalent must account for the barrier that accounts for the differential influence in tissue of the two ACE inhibitors used in this study.

We have demonstrated a graded dose-related inhibition of renal tissue Ang II formation by two different ACE inhibitors. Despite equal inhibition of the circulating hormonal system, ramipril inhibited the tissue intrarenal Ang II formation at a lower dose than did enalapril. ACE inhibitor doses 100-fold higher induced an identical maximal reduction in plasma and renal tissue Ang II concentrations that was still well above zero. Complete inhibition of Ang II formation cannot be achieved. Although the explanation for the difference at lower doses is not clear, the possibility that lipophilicity
20. Reams G, Villarreal D, Bauer JH. Intrarenal metabolism of angio-

21. Danzer JAH, Koning MMG, Admiral PJ, Sassen LMA, Derks
FHM, Verduin PD, Schalekamp MADH. Production of angiotensin I
and II at tissue sites in intact pigs. Am J Physiol. 1992;263:
H429-H437.

22. Admiral PJ, Danzer AHJ, Jong MS, Pieterman H, Derks FHM,
Schalekamp ADH. Regional angiotensin II production in essential
hypertension and renal artery stenosis. Hypertension. 1993;21:
173-184.

23. Phillips MI, Speeman EA, Kimura B. Levels of angiotensin and
molecular biology of the tissue renin angiotensin systems. Regul

24. Kifor I, Moore TJ, Fallo F, Sperling E, Menachery A, Chiou C-Y,
Williams GH. The effect of sodium intake on angiotensin content

25. Kifor I, Moore TJ, Fallo F, Sperling E, Chiou C-Y, Menachery A,
Williams GH. Potassium-stimulated angiotensin release from
superfused adrenal capsules and enzymatically dispersed cells of

TJ, Hollenberg NK. Independent and separate regulation of the
tissue and plasma angiotensin systems. Proceedings from the
Endocrine Society meeting, Las Vegas, Nev, June 9-12, 1993.


28. Campbell DJ, Kladis A. Simultaneous radioimmunoassay of six
angiotensin peptides in arterial and venous plasma of man. J
Hypertens. 1990;8:165-172.

29. Holtzman EJ, Braley LM, Williams GH, Hollenberg NK. Kinetics of
sodium homeostasis in rats: rapid excretion and equilibration

30. Hollenberg E, Braley LM, Menachery A, Williams GH, Hollenberg
NK. Rate of activation of renin-angiotensin-aldoctosterone axis and

immunoreactive angiotensin II in human plasma. Hypertension.

32. Richter C, Doussau MP, Giudicelli JF. Systemic and regional
hemodynamic profile of five angiotensin I converting enzyme
inhibitors in the spontaneously hypertensive rat. Am J Cardiol.
1987;59:123-17D.

33. Ranadive SA, Chen AX, Sarajudin ATM. Relative lipophilicities
and structural-pharmacological considerations of various angio-
tensin-converting enzyme (ACE) inhibitors. Pharmacol Res.


35. Cashman DW, Wang FL, Fung WC, Harvey C, DeForrest JM. Dif-
terentiation of angiotensin-converting enzyme (ACE) inhibitors
by their selective inhibition of ACE in physiologically important

36. Saikaguchi K, Chai SY, Jackson B, Johnston CI, Mendelsohn FAO.
F891-F898.


38. Ondetti MA. Structural relationships of angiotensin-converting
enzyme inhibitors to pharmacologic activity. J Hypertens.

tissue potencies of converting enzyme inhibitors: produg activa-

40. Ondetti MA. Structural relationships of angiotensin converting
enzyme inhibitors to pharmacologic activity. Circulation. 1988;77:
74-78.

41. Vasmanti D, Bender N. The renin-angiotensin system and ramipril,
1466-52.

42. Gohike P, Urbach B, Scholkens B, Unger T. Inhibition of con-
verting enzyme in the cerebrospinal fluid of rats after oral treat-
ment with converting enzyme inhibitors. J Pharmacol Exp
Ther. 1989;249:609-616.

Graf P, Brunner HR. Determinants of angiotensin II production
and structural-pharmacological considerations of various angio-
tensin-converting enzyme (ACE) inhibitors. Pharmacol Res.


45. Cashman DW, Wang FL, Fung WC, Harvey C, DeForrest JM. Dif-
terentiation of angiotensin-converting enzyme (ACE) inhibitors
by their selective inhibition of ACE in physiologically important

46. Saikaguchi K, Chai SY, Jackson B, Johnston CI, Mendelsohn FAO.
F891-F898.

Converting enzyme inhibition and renal tissue angiotensin II in the rat.
D R Allan, J A McKnight, I Kifor, C M Coletti and N K Hollenberg

Hypertension. 1994;24:516-522
doi: 10.1161/01.HYP.24.4.516

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/24/4/516

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/