The Origin of Urinary Angiotensins in Humans¹

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ABSTRACT
To examine whether urinary angiotensin (ANG) I and II excretion responds to changes in plasma ANG I and ANG II, ANG I or ANG II was infused in seven healthy subjects pretreated with a 340-mmol sodium diet and 20 mg of enalapril twice daily. Infusion rates were 4, 8, 16, and 32 pmol/kg per minute for ANG I and 1, 4, and 8 pmol/kg per minute for ANG II. Baseline ANG I and ANG II excretions averaged 10 and 20 fmol/min, respectively, which is approximately 0.3 and 5% of the filtered loads. Despite a 20-fold increase in plasma ANG I during ANG I infusion, urinary ANG I did not increase. Similarly, the 30-fold increase in plasma ANG II during ANG II infusion was not followed by an increase in ANG II excretion, but in fact by a decrease in urinary ANG I and ANG II. In a separate study, urinary ANG I and ANG II were measured before and after the oral administration of 20 mg of enalapril in eight healthy volunteers taking 400, 200, or 20 mmol of NaCl daily. In contrast to the considerable effects on plasma ANG I and ANG II and renal hemodynamics, enalapril had no effect on urinary ANG I and ANG II. Variation of sodium intake had predictable effects on plasma ANG I and ANG II but did not affect urinary ANG I and ANG II. These data suggest that urinary ANG I and ANG II originate from an intrarenal source. The independence of sodium intake and ANG-converting enzyme make the juxtaglomerular apparatus as the site responsible for the production of this ANG unlikely.

Key Words: Angiotensin, enalapril, intrarenal angiotensin system, sodium, urine

Evidence has been accumulating for the existence and importance of an intrarenal renin-angiotensin system (RAS) (1–14). Whole-kidney studies have demonstrated extensive de novo production of angiotensin (ANG) II (7). Measurements of ANG levels in kidney homogenates (2,13) and renal lymph (1,14) yielded much higher concentrations than in renal venous plasma. In histochemical studies, essential components of the RAS have been demonstrated in the juxtaglomerular apparatus (3). The production of ANG has been detected in juxtaglomerular cells (8). Functional evidence has been obtained for the production of ANG II in the proximal tubule (11,12) and for ANG I to ANG II conversion in the cortical interstitium (15). Finally, a high ANG degradation has also been documented at the whole-kidney level (10) and in proximal tubules (16,17).

Large discrepancies in activity have been reported between the systemic and intrarenal RAS under physiologic and pathophysiologic conditions, as emerged from studies on the activity of systemic and intrarenal RAS during variations in salt intake, ANG-converting enzyme (ACE) inhibitor administration (2,13), and experimental renovascular hypertension (18). A discrepancy between systemic RAS activity and urinary ANG excretion has also been demonstrated. Previous studies in our laboratory showed that increases in plasma ANG concentrations by postural changes did not coincide with changes in urinary ANG excretion (19). In contrast, thiazide administration increased ANG excretion with only minor changes in plasma ANG levels (19). In these and other studies in our laboratory (19,20), a relationship between the intrarenal RAS and urinary ANG excretion is suggested.

Methods to assess the intrarenal RAS in humans, which is of obvious importance in pathophysiology as well as in pharmacotherapy, are currently not available. In all of the above-mentioned studies, urinary ANG excretion was evaluated during maneuvers that concomitantly affected the systemic and intrarenal RAS. Consequently, it is still unclear to what extent urinary ANG excretion reflects changes in plasma ANG levels, rather than alterations in the activity of the intrarenal RAS. These experiments were performed to further delineate the usefulness of urinary ANG excretion as a method to assess renal RAS activity. In an attempt to assess the contribution of changes in plasma ANG levels to urinary ANG excretion in the absence of changes in the activity of the intrarenal RAS, urinary ANG excretion was evaluated during the iv infusion of ANG I or ANG II.

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Furthermore, ACE inhibitor was acutely administered to subjects at three levels of sodium intake to evaluate whether this maneuver, which supposedly diminishes both the systemic and intrarenal formation of ANG II, indeed resulted in alterations in urinary ANG excretion.

SUBJECTS AND METHODS

Two studies were carried out in healthy volunteers. Hypertension and other diseases were excluded by appropriate clinical and laboratory investigations. The protocols were approved by the Hospital Ethical Committee for Studies in Humans, and informed consent was obtained from each participant.

ANG I and II Infusion

Seven subjects (one woman, six men; age range, 20 to 25 yr) received a diet containing a high sodium content (340 mmol/day) for 5 consecutive days to suppress endogenous ANG I production. In addition, enalapril (20 mg twice daily) was taken to suppress ANG II formation. Diets were provided by the metabolic ward, where the subjects returned daily to deliver 24-h urine collections, to assess 24-h sodium excretion and adherence to the diet.

Clearance studies were performed on Days 5 and 6 of this regimen. A light carbohydrate breakfast was permitted at 7:00 a.m. Diuresis was ensured by an oral water load of 25 mL/kg body wt before studies were started and by the subsequent substitution of urinary water loss throughout the experiments. A iv infusion of inulin (10%) was administered throughout the studies to assess the GFR. After an equilibration period of 90 min and three baseline collection periods of 20 min each, the subjects received an infusion of either ANG I or ANG II. The order of the infusion (Day 5 or Day 6) was varied at random. The infusion rate of ANG I (synthetic human ANG I, Cambridge Research Biochemicals Ltd, Cambridge, United Kingdom; dissolved in 5% glucose in a concentration of 5 μg/mL) was increased in 30-min steps; the consecutive infusion rates were 4, 8, 16, and 32 pmol/kg per minute. During this infusion, urine was collected in 15-min portions. ANG II (synthetic human ANG II, Cambridge Research Biochemicals Ltd; dissolved in 5% glucose in a concentration of 1 μg/mL) was administered in a dosage of 1 pmol/kg for the first 30 min, followed by 4 pmol/kg during the next 30 min, and 8 pmol/kg per minute during the final 60 min. A longer infusion period during the highest dose was used because we expected a low urinary flow rate. During the highest infusion rate, the urine collection time was 30 min instead of 15 min. The urine samples were analyzed for volume and for inulin, ANG I, and ANG II concentrations. Blood specimens were obtained before the infusions and in the middle of each collection period and were assessed for plasma renin activity (PRA), aldosterone, ANG I, ANG II, and inulin.

Acute Administration of Enalapril at Different Levels of Sodium Intake

Eight subjects (one woman, seven men; age range, 20 to 25 yr) received diets containing 20, 200, and 400 mmol of NaCl per day in random order. Twenty-four-hour sodium excretion was measured to assess compliance to the diet. Each diet was continued for 4 days, and clearance studies were performed on Day 5 of each dietary period. A sustained infusion of inulin (10%) and para-aminobipyrurate (PAH; 2.5%) was administered for the measurement of GFR and estimated RPF. After an equilibration period of 90 min and three baseline urine collection periods of 20 min, 20 mg of enalapril was administered. The subjects were then studied for another six periods of 20 min. Urine collections for the determination of ANG I and II were taken during the second and third baseline collection periods and the fourth (60 to 80 min) and sixth (100 to 120 min) collection periods after the ingestion of enalapril. In the middle of each collection period, a blood sample was obtained as well. The blood samples were also assessed for PRA, plasma aldosterone, and ACE activity. Both urine and blood samples were assessed for inulin and PAH.

Analytical Methods

Plasma and urinary ANG I and ANG II (in picomoles per liter) were measured by radioimmunoassay (19). Blood was collected on ice with EDTA (final concentration in plasma, 15 mmol/L), Ro 42-5892 (2 μmol/L), captopril (100 μmol/L), o-phenantrolin (2 mmol/L), neomycin sulfate (0.3 mmol/L), and phenylmethane-sulfonyl fluoride (PMSF) (6 μmol/L), followed by centrifugation at 4°C immediately after collection to inhibit the activities of renin, ANG-converting enzyme, and angiotensinas. Urine samples were collected on ice with PMSF (3 μmol/L) to inhibit angiotensinase activity. Plasma and urine samples were chromatographed immediately after collection as described below. In this way, no degradation during the processing of plasma and urinary samples is detectable (complete recovery of added ANG). To assess the stability of ANG in urine, various amounts of ANG I or ANG II added to various urine specimens were incubated for 0, 1, 3, and 6 h at 0 and 37°C with and without the presence of PMSF. Urine ANG concentrations were between 5 and 20 fmol/mL and ANG amounted to 10 to 20 fmol per assay tube (see below). ANG were recovered completely after incubation with PMSF at 0 and 37°C up to 6 h and also without PMSF at 0°C. Incubation without PMSF at 37°C showed a gradual decline in ANG I and ANG II.
recoveries, the losses amounting to about 15, 40, and 60% after incubation for 1, 3, and 6 h, respectively. This indicates that some degradation is likely to occur in urine in the bladder, but because urine was collected in 15- to 30-min periods, the major part of the urinary ANG originally present is expected to be found in the assay. Urine and plasma extracts were stored at −80°C until the assay, at which temperature ANG are stable for 9 months.

ANG I and ANG II were extracted from 20 mL of urine (acidified to pH 3 with glacial acetic acid) or 2 mL of plasma (diluted with 8 mL of 0.035 mmol/L HCl) by solid phase extraction chromatography with a vacuum extraction device and butyl-silane extraction columns (Baker Chemical Co, Deventer, the Netherlands) and prewashed with methanol and deionized water. The effluents were discarded, and the columns were washed with 0.01 mmol/L HCl and eluted with methanol/trifluoroacetic acid (99:1 vol/vol). The eluate was dried and dissolved in assay buffer. Dilution curves of urine and plasma extracts, as well as standard curves containing aliquots of extract, were parallel to the standard curves used in the radioimmunoassay. Characteristics of the ANG I and ANG II assays are, respectively: sensitivity, 2 and 0.5 fmol; recovery of added standard material, in urine 72 and 89%; in plasma 73 and 90%, all at a level of 20 fmol/assay tube; the actual measurements were corrected for this percentage of recovery. Within-assay variation coefficients at a level of 20 fmol/assay tube are, respectively: for urine, 11 and 10%; for plasma, 9 and 10%; between-assay variation coefficients at this level are: for urine, 19 and 16%; for plasma, 17 and 19%.

For the ANG II radioimmunoassay, a specific antibody with very low cross-reactivity against ANG I and ANG II peptide fragments was used (Roche 923, courteously given by W. Fischli, F. Hoffmann-La Roche & Co, Basle, Switzerland) [21]. Cross-reactivities against ANG peptides are: ANG I < 0.4%: ANG2-a < 0.02%; ANG3-a < 0.02%; ANG4-a < 0.02%; ANG5-a < 0.02%. The addition of a mixture of ANG II peptide fragments and ANG I, resulting in the presence of 50 fmol of each peptide per assay tube, to urine and plasma samples with a low ANG II content (about 4 fmol/assay tube) did not increase the apparent immunoreactive ANG II concentration. Analysis of ANG II fractions obtained by high-performance liquid chromatography of these urine and plasma samples by the method previously described by Nussberger et al. [22] revealed concentrations similar to those obtained without high-performance liquid chromatography. Cross-reactivities of the ANG I antibody against ANG II and ANG II peptide fragments are negligible (<0.001%).

Inulin was measured photometrically with indoleacetic acid after hydrolyzation to fructose [23]. PAH was determined photometrically by a chromogenic aldehyde reaction [24]. PRA (in femtomoles of ANG I per liter per second) and plasma aldosterone (in picoles per liter) were measured by radioimmunoassay [25,26]. ACE activity (in micromoles per liter per second) was measured by a colorimetric method by hydrolysis of the synthetic substrate L-Hip-His-Leu [27].

Calculations and Statistics

Urinary excretion, clearances, and fractional clearances were calculated by the use of standard formulae. Data are presented as mean ± SE. Plasma ANG I and II, PRA, plasma aldosterone, and filtered load are presented as geometric means, because they are not normally distributed. Statistical analysis of these variables was performed on the logarithmically converted data. Statistical analysis of data from the ANG infusion experiments was performed by one-way analysis of variance for repeated measures (ANOVA). The data obtained from the study on acute enalapril administration during different levels of sodium intake were analyzed by a two-way, randomized block design ANOVA. If the variance ratio obtained by ANOVA reached statistical significance, the differences between the means of observations were analyzed by the method of the least significant difference [28], with the ANOVA mean square error (pooled error variance) used to calculate the least significant difference at the 5% significance level.

RESULTS

ANG I and II Infusion

Compliance to the diet appears from the 24-h urine sodium excretion, which amounted to 324 ± 28 and 333 ± 19 mmol on Days 3 and 4 of the dietary regimen, respectively. Substantial reduction of ACE activity was achieved by the oral administration of enalapril, as plasma ACE activity was 0.05 ± 0.03 μmol/L per second during the third baseline collection period and 0.05 ± 0.02 μmol/L per second at the end of the clearance study (normal range, 0.30 to 1.20 μmol/L per second).

In the baseline period of the ANG I infusion protocol, the ANG I excretion rate was 10 ± 2 fmol/min and the fractional excretion was only 0.3 ± 0.2%. Both absolute and fractional excretion rates of ANG II were higher than those of ANG I during the baseline period and averaged 22 ± 3 fmol/min and 5.4 ± 0.8%, respectively. ANG I infusion caused a significant reduction in GFR and urine flow. Each stepwise increase in the infusion rate of ANG I was followed by an increase in plasma ANG I so that the final ANG I concentration was increased about 20 times as compared with the baseline value. However, the urinary
excretion of ANG I did not change. Despite the pre-treatment with the ACE inhibitor enalapril, the ANG I infusion was followed by a rise in plasma ANG II. The increased filtered load of ANG II coincided with a significant decrease in the absolute excretion of ANG II at infusion rates of 16 and 32 pmol/kg per minute. The ANG I infusion had no significant effects on PRA. Plasma aldosterone increased modestly at the highest ANG I infusion rate only. Data from the ANG I infusion protocol are summarized in Table 1 and Figure 1.

The infusion of ANG II also caused a fall in GFR and urine flow. The decrease in both plasma ANG I and filtered load of ANG I was accompanied by a decrease in the absolute ANG I excretion rate. Plasma ANG II increased 30-fold as compared with baseline values. The substantially increased filtered load of ANG II was accompanied by a significant decrease in the absolute excretion rate of both ANG I and ANG II at the ANG II infusion rate of 8 pmol/kg per minute. The decrease in absolute ANG II excretion was more pronounced than the decrease observed in the ANG I infusion experiments. Predictably, ANG II infusion suppressed PRA and increased plasma aldosterone levels. Table 2 and Figure 2 summarize the data obtained from the ANG II infusion experiments.

**Acute Administration of Enalapril at Different Levels of Sodium Intake**

Adherence to the three diets is apparent from body weight and 24-h sodium excretions. Body weight was 77.3 ± 5.3, 76.0 ± 5.2, and 75.5 ± 5.2 kg during the 400, 200, and 20 mmol of NaCl/day diets, respectively. Sodium excretion averaged 367 ± 11, 205 ± 15, and 25 ± 3 mmol/24 h during the respective diets. The changes in dietary sodium had predictable effects on the systemic renin-ANG-aldosterone system: PRA, plasma aldosterone, ANG I, and ANG II were stimulated by the low-sodium diet and suppressed by the high-sodium diet (compare baseline values in Tables 3, 4, and 5). Despite the large vari-

![Graph](attachment:image.png)

**Figure 1.** Plasma ANG concentration and urinary ANG excretion during the ANG I infusion experiments. *P < 0.05 compared with baseline values (ANOVA, least significant difference test).

**TABLE 1.** Plasma and urinary ANG I and II, PRA, and plasma aldosterone during ANG I infusion

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4 (pmol/kg per minute)</th>
<th>8 (pmol/kg per minute)</th>
<th>16 (pmol/kg per minute)</th>
<th>32 (pmol/kg per minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inulin Clearance</strong></td>
<td>134 ± 8</td>
<td>131 ± 8</td>
<td>131 ± 8</td>
<td>128 ± 7</td>
<td>119 ± 8b</td>
</tr>
<tr>
<td><strong>Urine Flow</strong></td>
<td>21 ± 2</td>
<td>19 ± 2</td>
<td>19 ± 2</td>
<td>17 ± 2ab</td>
<td>14 ± 2ab</td>
</tr>
<tr>
<td><strong>Plasma ANG I</strong></td>
<td>26 ± 18</td>
<td>67 ± 23a</td>
<td>117 ± 42a</td>
<td>229 ± 54ab</td>
<td>578 ± 120ab</td>
</tr>
<tr>
<td><strong>Filtered Load of ANG I</strong></td>
<td>4 ± 3</td>
<td>6 ± 3</td>
<td>15 ± 4c</td>
<td>30 ± 8c</td>
<td>49 ± 16ac</td>
</tr>
<tr>
<td><strong>Urine ANG I Excretion</strong></td>
<td>10 ± 2</td>
<td>8 ± 2</td>
<td>8 ± 2</td>
<td>8 ± 2</td>
<td>8 ± 2</td>
</tr>
<tr>
<td><strong>Plasma ANG II</strong></td>
<td>2.9 ± 0.5</td>
<td>4.1 ± 0.8</td>
<td>7.9 ± 3ab</td>
<td>15 ± 3ab</td>
<td>29 ± 5ab</td>
</tr>
<tr>
<td><strong>Filtered Load of ANG II</strong></td>
<td>0.40 ± 0.08</td>
<td>0.5 ± 0.2</td>
<td>1.2 ± 0.3ab</td>
<td>2.2 ± 0.5ab</td>
<td>3.5 ± 0.6ab</td>
</tr>
<tr>
<td><strong>Urine ANG II Excretion</strong></td>
<td>22 ± 3</td>
<td>18 ± 3</td>
<td>20 ± 4</td>
<td>16 ± 2ab</td>
<td>15 ± 3a</td>
</tr>
<tr>
<td><strong>PRA</strong></td>
<td>225 ± 160</td>
<td>210 ± 90</td>
<td>180 ± 70</td>
<td>180 ± 50</td>
<td>210 ± 40</td>
</tr>
<tr>
<td><strong>Plasma Aldosterone</strong></td>
<td>140 ± 30</td>
<td>130 ± 40</td>
<td>150 ± 50</td>
<td>160 ± 50</td>
<td>210 ± 80a</td>
</tr>
</tbody>
</table>

* P < 0.05 versus baseline.

b P < 0.05 versus preceding dose of ANG I.

P < 0.05 versus 8 pmol/kg of ANG I per minute.

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TABLE 2. Plasma and urinary ANG I and II, PRA, and plasma aldosterone during ANG II infusion

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>ANG II Infusion (pmol/kg per minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Inulin Clearance (mL/min)</td>
<td>138 ± 9</td>
<td>128 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urine Flow (mL/min)</td>
<td>21 ± 2</td>
<td>13 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma ANG I (pmol/L)</td>
<td>35 ± 21</td>
<td>17 ± 12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Filtered Load of ANG I (pmol/min)</td>
<td>4.6 ± 2.6</td>
<td>2.2 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urine ANG I Excretion (fmol/min)</td>
<td>11 ± 3</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Plasma ANG II (pmol/L)</td>
<td>3 ± 2</td>
<td>12 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Filtered Load of ANG II (pmol/min)</td>
<td>0.38 ± 0.06</td>
<td>1.5 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urine ANG II Excretion (fmol/min)</td>
<td>20 ± 4</td>
<td>25 ± 9</td>
</tr>
<tr>
<td>PRA (fmol/L per second)</td>
<td>280 ± 160</td>
<td>210 ± 110&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma Aldosterone (pmol/L)</td>
<td>110 ± 30</td>
<td>150 ± 40</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.05 versus baseline.
<sup>b</sup> P < 0.05 versus dose of ANG II.
<sup>c</sup> P < 0.05 versus 1 pmol/kg of ANG II per minute.

Plasma ANG I and decreased plasma ANG II levels. This effect was most pronounced during the lowsodium diet. Nonetheless, the urinary excretion of these peptides did not change. The effectiveness of systemic ACE inhibition can be appreciated from the consistent decrease in plasma ACE activity. Furthermore, in agreement with the known effects of ACE inhibition on renal hemodynamics, filtration fraction decreased.

DISCUSSION

This study was designed to further explore the urinary excretion of ANG I and ANG II as an indicator of the activity of the intrarenal RAS. The main finding is that substantial elevations of plasma ANG I or ANG II by means of ANG I or ANG II infusion failed to increase the urinary excretion of these peptides but, in contrast, resulted in a decrease in the urinary excretion of ANG II. Neither chronic alterations in sodium intake nor acute ACE inhibitor administration resulted in discernable changes in ANG I and ANG II excretion.

Under baseline conditions, the fraction of excreted ANG I and ANG II approximated 0.5 and 5% of the filtered load, respectively. Previous studies have reported even lower recovery of urinary ANG II in microinfusion experiments in rat superficial proximal tubules (17). Remarkably, the excretion of ANG I was considerably less than that of ANG II. The failure to show a positive relationship between the 10- to 30-fold increased filtered load of ANG I and ANG II and urinary ANG excretion makes it unlikely that urinary ANG are derived from a systemic source. Furthermore, both the low excretion under baseline conditions and the data from the infusion experiments are in agreement with the notion that the kidney is ca-
pable of degrading or reabsorbing large quantities of small peptides, in particular, ANG II (16,17).

It is hard to conceive that increased proximal tubular degradation solely is responsible for the observation that urinary ANG II excretion decreased during ANG I and ANG II infusion. Microperfusion experiments in rats have demonstrated an almost linear relationship between perfused ANG II and degraded ANG II over a wide range of loads (17). Furthermore, it has been demonstrated that ANG II microinfused into the distal nephron is excreted almost completely unaltered in the urine (16). An increase in proximal tubular reabsorption by ANG II (15) could lead to a lower proximal flow and the stimulation of the proximal tubular degradation of ANG II, so that less ANG II reaches the distal nephron and is excreted in the urine. However, it is not likely that the proximal tubule will overcompensate the increased filtered load. Because the degradation of filtered ANG II is insufficient to explain the observations, the inhibition of an intrarenal production site should be considered. The existence of a negative feedback between ANG II and the RAS has been well documented (29–31) and was recently further established by the

### TABLE 3. Renal hemodynamics, plasma and urinary ANG I and II, PRA, plasma aldosterone, and ACE before and 80 and 120 min after the oral administration of 20 mg of enalapril during a 400-mmol NaCl diet

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Enalapril 80 min</th>
<th>Enalapril 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inulin Clearance (mL/min)</td>
<td>136 ± 9</td>
<td>130 ± 10</td>
<td>130 ± 8</td>
</tr>
<tr>
<td>PAH Clearance (mL/min)</td>
<td>700 ± 50</td>
<td>710 ± 50</td>
<td>740 ± 40</td>
</tr>
</tbody>
</table>
| Filtration Fraction (%) | 18.6 ± 0.7 | 18.7 ± 0.9 | 17.9 ± 0.8
| Plasma ANG I (pmol/L) | 15 ± 3 | 18 ± 4 | 21 ± 5
| Filtered Load of ANG I (pmol/min) | 2.0 ± 0.3 | 2.3 ± 0.4 | 2.7 ± 0.6
| Urine ANG I Excretion (fmol/min) | 11 ± 2 | 11 ± 2 | 10 ± 1
| Plasma ANG II (pmol/L) | 4.5 ± 0.5 | 4.0 ± 0.8 | 2.4 ± 0.4
| Filtered Load of ANG II (pmol/min) | 0.60 ± 0.07 | 0.51 ± 0.09 | 0.31 ± 0.05
| Urine ANG II Excretion (fmol/min) | 24 ± 5 | 25 ± 5 | 21 ± 5
| PRA (fmol/L per minute) | 85 ± 20 | 80 ± 20 | 95 ± 35
| Plasma Aldosterone (pmol/L) | 145 ± 30 | 90 ± 15 | 80 ± 15
| ACE Activity (µmol/L per second) | 0.75 ± 0.09 | 0.19 ± 0.06 | 0.10 ± 0.03

* P < 0.05 versus baseline.
* P < 0.05 versus preceding period.

### TABLE 4. Renal hemodynamics, plasma and urinary ANG I and II, PRA, plasma aldosterone, and ACE before and 80 and 120 min after the oral administration of 20 mg of enalapril during a 200-mmol NaCl diet

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>Enalapril 80 min</th>
<th>Enalapril 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inulin Clearance (mL/min)</td>
<td>132 ± 9</td>
<td>132 ± 4</td>
<td>133 ± 7</td>
</tr>
<tr>
<td>PAH Clearance (mL/min)</td>
<td>710 ± 40</td>
<td>740 ± 50</td>
<td>760 ± 50</td>
</tr>
</tbody>
</table>
| Filtration Fraction (%) | 18.6 ± 0.8 | 18.1 ± 0.9 | 17.7 ± 0.9
| Plasma ANG I (pmol/L) | 31 ± 7* | 30 ± 10 | 40 ± 20
| Filtered Load of ANG I (pmol/min) | 4.0 ± 0.9* | 3.9 ± 0.3 | 5 ± 3
| Urine ANG I Excretion (fmol/min) | 9 ± 1 | 10 ± 2 | 10 ± 2
| Plasma ANG II (pmol/L) | 11 ± 3* | 7 ± 1 | 4.2 ± 0.6
| Filtered Load of ANG II (pmol/min) | 1.5 ± 0.4* | 1.0 ± 0.2 | 0.54 ± 0.07
| Urine ANG II Excretion (fmol/min) | 17 ± 3 | 21 ± 4 | 21 ± 5
| PRA (fmol/L per minute) | 250 ± 85 | 265 ± 100 | 340 ± 160
| Plasma Aldosterone (pmol/L) | 230 ± 50 | 155 ± 15 | 125 ± 25
| ACE Activity (µmol/L per second) | 0.67 ± 0.08 | 0.19 ± 0.03 | 0.10 ± 0.02

* P < 0.05 versus same period during 400-mmol NaCl diet.
* P < 0.05 versus baseline.
* P < 0.05 versus preceding study period.
demonstration of decreased renin mRNA expression in the kidneys of rats subjected to ANG II infusion (32) and increased juxtaglomerular renin mRNA expression during ACE inhibition (33). Support for the suppression of an intrarenal source of ANG is formed by our finding of a concomitant decrease of ANG I and ANG II excretion in the ANG II infusion experiments and is consistent with the decrease in PRA, indicating the suppression of renin release.

The second protocol evaluated the effect of the acute inhibition of ANG I to ANG II conversion with the ACE inhibitor enalapril on urinary ANG excretion. In particular during the low-sodium diet, enalapril resulted in pronounced stimulation of PRA and plasma ANG I and suppression of ANG II in blood samples obtained at 80 and 120 min after ingestion. Although peak changes may not be reached until later, similar early changes in the systemic activity of the RAS were reported by other investigators (34–36). In spite of these effects, no changes in urinary ANG excretion rate were observed. It was anticipated that acute ACE inhibitor administration would affect intrarenal ANG conversion in particular during a low-sodium intake, because this maneuver is expected to stimulate both systemic and intrarenal RAS activity. Indeed, the low-sodium diet increased basal PRA and plasma ANG I and II and the administration of enalapril was followed by a significant increase in RPF. Although this indicates that the dosage of enalapril was sufficient to affect renal hemodynamics, no change in the urinary excretion of ANG I or ANG II was observed. This indicates that the compartment responsible for the production of ANG I and ANG II excreted with the urine is not accessible to acute enalapril administration or is independent of ACE activity. Similar to the results of acute ACE inhibition, changes in sodium intake did not affect the urinary excretion of ANG, despite predictable changes in the systemic RAS.

As mentioned, this demonstration that the excretion of urinary ANG I and II is fully independent of the plasma concentrations of these peptides suggests that urinary ANG are of intrarenal origin. Because the excretion of these peptides does not depend on sodium intake, their source does not seem to be controlled by volume-regulating mechanisms. Moreover, the production is probably not influenced by the administration of an ACE inhibitor. By contrast, morphologic and histochemical studies have indicated that RAS activity in juxtaglomerular cells is both volume controlled (37–39) and sensitive to oral ACE inhibitor (32,39,40). Altogether, these findings make it unlikely that the juxtaglomerular apparatus is the intrarenal production site of angiotensin excreted in the urine.

A possible alternative production site concerns the proximal tubules. Histochemical studies have shown that, besides the juxtaglomerular apparatus (8), the proximal tubule possesses essential components of the RAS (9,41,42). In situ hybridization studies have demonstrated predominant angiotensinogen mRNA expression in the proximal tubule (9). This may explain the recent finding of 1,000-fold higher ANG II concentrations in the proximal tubule than in plasma (11,12), strongly suggesting local production of ANG II. Interestingly, a recent communication has shown that, in rats pretreated with the ACE inhibitor quinapril, the renin mRNA in glomerular cells is up-

### TABLE 5. Renal hemodynamics, plasma and urinary ANG I and II, PRA, plasma aldosterone, and ACE before and 80 and 120 min after the oral administration of 20 mg of enalapril during a 20-mmol NaCl diet

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Enalapril 80 min</th>
<th>Enalapril 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inulin Clearance (mL/min)</td>
<td>126 ± 6a</td>
<td>120 ± 8a</td>
<td>123 ± 6a</td>
</tr>
<tr>
<td>PAH Clearance (mL/min)</td>
<td>720 ± 70</td>
<td>760 ± 80b</td>
<td>810 ± 80c</td>
</tr>
<tr>
<td>Filtration Fraction (%)</td>
<td>18.3 ± 1.4</td>
<td>17.0 ± 1.5ab</td>
<td>16.0 ± 1.2ac</td>
</tr>
<tr>
<td>Plasma ANG I (pmol/l)</td>
<td>58 ± 8a</td>
<td>120 ± 70b</td>
<td>170 ± 70ac</td>
</tr>
<tr>
<td>Filtered Load of ANG I (pmol/min)</td>
<td>7.2 ± 0.8</td>
<td>14 ± 5ab</td>
<td>21 ± 6ac</td>
</tr>
<tr>
<td>Urine ANG I Excretion (fmol/min)</td>
<td>11 ± 2</td>
<td>11 ± 2</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>Plasma ANG II (pmol/l)</td>
<td>22 ± 5a</td>
<td>5 ± 2ab</td>
<td>3.6 ± 0.8ab</td>
</tr>
<tr>
<td>Filtered Load of ANG II (pmol/min)</td>
<td>2.8 ± 0.7a</td>
<td>0.6 ± 0.2ab</td>
<td>0.36 ± 0.07ac</td>
</tr>
<tr>
<td>Urine ANG II Excretion (fmol/min)</td>
<td>21 ± 5</td>
<td>18 ± 6</td>
<td>22 ± 5</td>
</tr>
<tr>
<td>PRA (fmol/L per minute)</td>
<td>580 ± 130a</td>
<td>860 ± 380ab</td>
<td>1450 ± 700ab</td>
</tr>
<tr>
<td>Plasma Aldosterone (pmol/L)</td>
<td>425 ± 80a</td>
<td>205 ± 55ad</td>
<td>175 ± 49ad</td>
</tr>
<tr>
<td>ACE Activity (μmol/L per second)</td>
<td>0.68 ± 0.08</td>
<td>0.12 ± 0.03b</td>
<td>0.07 ± 0.02b</td>
</tr>
</tbody>
</table>

* P < 0.05 versus same period during both 400- and 200-mmol NaCl diets.

* P < 0.05 versus baseline.

* P < 0.05 versus previous period.

* P < 0.05 versus same period during 400-mmol NaCl diet.
regulated, whereas renin mRNA in proximal tubules is not affected (33). Moreover, the perfusion of proximal tubules with enalaprilate did not alter the high ANG II concentrations in proximal tubular fluid, suggesting that ANG II production is independent of ACE or inaccessible to an ACE inhibitor (12). It has also been demonstrated that the amount of ANG II in the proximal tubular fluid is not different in volume-expanded versus hydropenic rats (12). In the face of these observations, the urinary ANG excretion, which also appears ACE and volume independent, may well be derived from ANG formed in or produced by the proximal tubules. Our previous observation that chlorothiazide infusion stimulates urinary ANG excretion while decreasing plasma ANG I and II (19) is also compatible with this idea of a proximal tubular origin of urinary ANG.

A problem remains that the recovery of urinary ANG is low relative to the amounts filtered in the glomeruli or produced along the proximal tubules. However, the proximal tubules are also capable of the rapid degradation of ANG (16,17), and the amounts escaping this segment may be very small and account for the low urinary ANG excretion rates. Because the subjects were not catheterized, some intraturinary peptide degradation probably occurred. However, in view of the short urine collection periods and the quick processing of the urine samples, the intraturinary degradation of ANG I and II was limited to at most a few percents of the amounts excreted by the kidneys (see Methods) and cannot account for the low urinary excretion rates.

Our observation that urinary ANG excretion in humans is not influenced by ACE inhibition not only makes the juxtaglomerular apparatus unlikely as a production site, but also indicates that urinary ANG II excretion cannot be used as a marker for the efficacy of the suppression of the renal RAS. Future studies are necessary to clarify whether urinary ANG are useful as a marker of tubular ANG production.

ACKNOWLEDGMENTS

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REFERENCES


