Nephrectomy, Converting Enzyme Inhibition, and Angiotensin Peptides

Duncan J. Campbell, Athena Kladis, Ann-Maree Duncan

To determine the contribution of kidney-derived renin and angiotensin converting enzyme to circulating and tissue levels of angiotensin peptides, we measured angiotensin (Ang)-(1-7), Ang II, Ang-(1-9), and Ang I in plasma, kidney, lung, heart, aorta, brown adipose tissue, adrenal, pituitary, and brain of five groups of male Sprague-Dawley rats: control rats, rats given the converting enzyme inhibitor ramipril (10 mg/kg), rats nephrectomized 24 hours, rats nephrectomized 48 hours, and rats nephrectomized 48 hours and given ramipril. Plasma and tissues, apart from adrenal, showed a 63% to 98% reduction in Ang II, the ratio of Ang II to Ang I, or both after ramipril administration, indicating a major role for converting enzyme in Ang II formation. Nephrectomy caused a more than 95% decrease in plasma renin levels and a fourfold to eightfold increase in plasma angiotensinogen levels. Apart from plasma and brain, tissues showed a 59% to 78% decrease in Ang II levels after nephrectomy, indicating a major role for kidney-derived renin in Ang II formation. The persistence of Ang II in plasma and tissues of anephric rats indicates that Ang II may be formed by a process independent of kidney-derived renin; this process may be amplified by the increased plasma angiotensinogen levels that accompany nephrectomy. For lung, adrenal, and aorta, Ang II levels showed a further decrease when nephrectomized rats were given ramipril. However, for plasma and the other tissues, ramipril produced little or no decrease in Ang II levels of anephric rats, suggesting that Ang II may be formed by a pathway independent of converting enzyme. Such a pathway may involve the direct formation of Ang II from angiotensinogen by a non-reninlike enzyme. (Hypertension. 1993;22:513-522.)

Key Words • angiotensin II • angiotensin I • radioimmunoassay • chromatography, high-performance liquid

Angiotensin II (Ang II) plays a major role in the regulation of blood pressure and fluid and electrolyte homeostasis. In addition to the circulating renin-angiotensin system, a large body of evidence indicates that tissues are a major site of Ang II formation and that they make an important contribution to circulating levels of angiotensin I (Ang I) and Ang II. Despite the important role of tissues in angiotensin peptide formation, little is known of the mechanism by which tissue production of angiotensin peptides occurs. Angiotensin production in tissues may involve one or more of several mechanisms. Tissue production of angiotensin peptides may result from the local synthesis of renin and angiotensinogen. In support of this possibility, the mRNA for renin and angiotensinogen has been detected in many tissues (reviewed in Reference 2). However, renin mRNA codes for the inactive prorenin and the extent to which extrarenal tissues activate prorenin are unknown. Tissue production of angiotensin peptides may also result from the uptake of renin and angiotensinogen from plasma. There is evidence for the vascular uptake of circulating renin, and the angiotensinogen concentration of interstitial fluid may approximate that of plasma. Another possibility is tissue production of angiotensin peptides by a combination of these two mechanisms, whereby locally synthesized renin acts on plasma-derived angiotensinogen, or plasma-derived (kidney-derived) renin acts on locally synthesized angiotensinogen.

In addition to the classic pathway of Ang II formation involving renin and angiotensin converting enzyme (ACE), alternative pathways may operate in tissues (Fig 1). Angiotensinogen may be cleaved by aspartyl proteases other than renin to release Ang I and by serine proteases to release Ang II directly (reviewed in Reference 9). Moreover, Ang I may be converted to Ang II by serine protease activity or by the sequential cleavage of the two carboxy terminal residues of Ang I by carboxypeptidase activity.

In the present study, we investigated the role of kidney-derived renin and ACE in angiotensin production in plasma and tissues of the rat by applying high-performance liquid chromatography (HPLC)-based radioimmunoassays to the measurement of four angiotensin peptides in plasma and tissues: angiotensin-(1-7) [Ang-(1-7)], Ang II, angiotensin-(1-9) [Ang-(1-9)], and Ang I. We studied the effects on angiotensin peptide levels of ACE inhibition, of bilateral nephrectomy, and of ACE inhibition in anephric rats. These studies demonstrate that tissue angiotensin peptides result from local production and that the major mechanism of angiotensin peptide production in plasma and tissues involves kidney-derived renin and ACE. These studies also provide evidence for the operation of alternative pathways of angiotensin pep-


**Methods**

**Animals**

Male Sprague-Dawley rats (250 to 300 g), maintained in a room with a 12-hour light/dark cycle (lights on 6 AM to 6 PM), were fed a diet of GR 2+ pellets (Clarke King & Co, Melbourne, Australia) and received tap water to drink. These studies were performed in accordance with the guidelines of St Vincent's Hospital Animal Experimentation Ethics Committee.

Five groups of rats were studied: A, control rats; B, rats administered the ACE inhibitor ramipril (10 mg/kg) by intraperitoneal injection 4 hours before death; C, rats nephrectomized 24 hours before death; D, rats nephrectomized 48 hours before death; and E, rats nephrectomized 48 hours before and had received ramipril (10 mg/kg) by intraperitoneal injection 4 hours before were killed by decapitation without prior anesthesia. Rats were killed by decapitation without prior anesthetic (unless specified otherwise). Ramipril was a generous gift from Hoechst Australia Ltd, Melbourne, Australia.

**Extraction and Radioimmunoassay of Angiotensin Peptides From Plasma**

Trunk blood (5 to 6 mL) was rapidly collected into tubes containing 0.5 mL inhibitor solution (1 mmol/L SQ 30697, 146 μmol/L peptatin, 50 mmol/L 1,10-phenanthroline, 125 mmol/L EDTA, 2 g/L neomycin sulfate, 2% dimethyl sulfoxide, and 2% ethanol in water) at 4°C. The renin inhibitor SQ 30697 was a generous gift from Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ. The final plasma concentration of SQ 30697 (100 to 200 μmol/L) was sufficient to cause complete inhibition of rat renin (unpublished data from our laboratory). The blood was centrifuged and the plasma (approximately 3 mL) was immediately extracted with Sep-Pak C18 cartridges (Waters Chromatography Division, Milford, Mass) as described in detail elsewhere. Angiotensin peptides were acetylated before HPLC, and after reconstitution in water, each HPLC fraction was assayed in duplicate with the amino terminal-directed antibody A41, which measures acetyl-Ang-(1-7), acetyl-Ang II, acetyl-Ang-(1-9), and acetyl-Ang I. Acetylation and HPLC were performed using a modification of the method previously described. The two plasma samples were processed in parallel, and recoveries were calculated by subtracting the endogenous peptide levels and expressing the result as a percentage of the amount added.

**Extraction and Radioimmunoassay of Angiotensin Peptides From Tissues**

Kidney, lung, heart (cardiac ventricles), aorta, peri-aortic brown adipose tissue with associated connective tissue, adrenals, and brain (comprising brain stem, hypothalamus, thalamus, septum, and midbrain) were rapidly removed, weighed, and immediately homogenized in 4 mol/L guanidine thiocyanate (GTC)/1% trifluoroacetic acid (TFA) (vol/vol) in water and then processed as described previously before HPLC and measurement of angiotensin peptides by radioimmunooassay as described for plasma. Each pituitary sample contained six to 11 pituitaries, which were individually sonicated on removal in 1 mL GTC/TFA and then pooled before processing as described for the other tissues. Given the number of rats required for collection of pituitaries, for most rats only blood or three to four tissues, in addition to pituitary, were collected from each rat within 2 to 3 minutes of decapitation.

For each of six estimations of recovery of angiotensin peptides from GTC/TFA homogenates of each tissue, 20 mL kidney homogenate or 20 mL pooled homogenate of other tissues was divided into two equal portions, to one of which was added 50 μL of the peptide cocktail described above. The samples were processed in parallel and the peptide recoveries calculated as described for plasma.

**Extraction and Radioimmunoassay of Angiotensin Peptides From Blood**

Angiotensin peptides were also measured in whole blood collected from conscious unrestrained rats. For these experiments, rats were anesthetized with sodium pentobarbital, and carotid arterial cannulas were inserted and exteriorized at the back of the neck. The cannulas were filled with heparinized saline (20 IU/mL). For collection of blood from anephric rats, nephrectomy was performed at the time of cannulation of the carotid artery. Twenty-four or 48 hours later, 2 mL blood was collected from the cannulas directly into syringes containing 10 mL GTC/TFA. The blood and GTC/TFA were then mixed, transferred to a 50-mL tube, homogenized briefly, and processed as described above for tissues. Ang-(1-9) was not measured in these samples; instead, bradykinin was measured as described previously.

In a separate experiment, rats that had been nephrectomized 48 hours before and had received ramipril (10 mg/kg) by intraperitoneal injection 4 hours before were anesthetized with ether, the abdominal aorta exposed, and 4 mL blood rapidly collected containing 20 mL GTC/TFA. The blood and GTC/TFA were then processed as described above. For one of these samples, HPLC fractions were assayed with both the 

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**Diagagrammatic representation shows alternative pathways of angiotensin II formation from angiotensinogen. ACE, angiotensin converting enzyme.**

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**Fig 1.**
Angiotensin Peptides in Plasma, Blood, and Tissues of Angiotensin Converting Enzyme in Plasma

values were below the minimum detectable, they were

HPLC) and minimum detectable amounts of angioten-

Control Rats


was that converting enzyme inhibition and nephrectomy

would reduce these levels, and comparisons with control

were examined by Dunnett's one-tailed test (mean<

than half of the samples comprising a mean had values

below the minimum detectable, the sample mean is

shown as less than the minimum detectable. Data were

analyzed by analysis of variance of the five groups of rats

(A through E). For Ang II levels, the a priori hypothesis

was that converting enzyme inhibition and nephrectomy

would reduce these levels, and comparisons with control

were examined by Dunnett's one-tailed test (mean<

control). For other parameters [Ang-(1-7), Ang-(1-9),

Ang I, Ang II–Ang I ratio, renin, angiotensinogen, and

ACE], comparisons with control were examined with Dunnett's two-tailed test. Other comparisons between

groups were made with the Games-Howell test. When

values were below the minimum detectable, they were

set at half the minimum detectable for statistical calcula-

tions. Logarithmic transformation of data was performed

when appropriate to obtain similar variances among

groups. Analyses were performed with SUPER-


Results

Angiotensin Peptides in Plasma, Blood, and Tissues of Control Rats

The recoveries (from extraction, acetylation, and

HPLC) and minimum detectable amounts of angioten-

sin peptides in plasma, blood, and tissues are shown in

Table 1. All data have been corrected for recovery and cross-reactivity with antisera.

Angiotensin peptides were detected in all tissues studied (Fig 2). Whereas Ang II and Ang I levels were similar for plasma, Ang II was the predominant angiotensin peptide in tissues, with marked variation in Ang II levels between tissues (Tables 2 and 3). All tissues had higher Ang II–Ang I ratios than plasma. For most tissues, Ang-(1-7) and Ang-(1-9) levels were at or below the minimum detectable.

Effect of Nephrectomy and Ramipril on Plasma Renin, Angiotensinogen, and Angiotensin Converting Enzyme

Nephrectomy caused a marked fall in plasma renin levels to less than 2% of control at 48 hours, with an associated fourfold to eightfold increase in plasma angiotensinogen levels and a 57% increase in plasma ACE activity at 48 hours (Table 4). Ramipril caused a 23-fold increase in plasma renin levels of intact rats, with an associated 60% fall in plasma angiotensinogen levels, and plasma ACE activity was reduced to less than 5% of control. By contrast, ramipril had no effect on either renin or angiotensinogen levels of anephric rats, but plasma ACE activity was again maximally suppressed.

Effect of Ramipril on Angiotensin Peptides in Plasma and Tissues of Intact Rats

Ramipril caused a 22-fold increase in plasma Ang I levels of intact rats, similar to the increase in plasma renin, with a 13-fold increase in Ang-(1-7) but no change in Ang-(1-9) levels (Table 2). Ang II fell to 35% of control levels, and the Ang II–Ang I ratio fell to 2% of control (Table 2, Fig 3), in agreement with the marked suppression of plasma ACE activity by ramipril.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recoveries, %</th>
<th>Minimum Detectable Levels, fmol/mL or fmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ang-(1-7)</td>
<td>Ang II</td>
</tr>
<tr>
<td>Plasma</td>
<td>31±12</td>
<td>41±10</td>
</tr>
<tr>
<td>Blood</td>
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<td>51±11</td>
</tr>
<tr>
<td>Kidney</td>
<td>33±9</td>
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<tr>
<td>Lung</td>
<td>37±8</td>
<td>46±13</td>
</tr>
<tr>
<td>Heart</td>
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<td>50±6</td>
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<tr>
<td>Aorta</td>
<td>37±6</td>
<td>49±10</td>
</tr>
<tr>
<td>Brown adipose tissue</td>
<td>39±4</td>
<td>55±12</td>
</tr>
<tr>
<td>Adrenal</td>
<td>40±10</td>
<td>53±7</td>
</tr>
<tr>
<td>Brain</td>
<td>19±5</td>
<td>34±7</td>
</tr>
<tr>
<td>Pituitary</td>
<td>40±8</td>
<td>44±19</td>
</tr>
</tbody>
</table>

Ang indicates angiotensin. Data are mean±SD; n=6 for plasma, blood, and all tissues except pituitary, where n=4. Calculation of minimum detectable levels was based on minimum peak height of 0.5 fmol for aorta and brown adipose tissue and 1.0 fmol for plasma, blood, and other tissues and includes correction for peptide recovery, antibody cross-reactivity, and sample size. Minimum detectable levels apply to mean plasma volume of 2.7 mL, blood volume of 2 mL, and mean wet weight of 0.86 g for kidney, 0.75 g for lung, 0.83 g for heart, 0.075 g for aorta, 0.195 g for brown adipose tissue, 0.054 g for adrenal, 0.49 g for brain, and 0.07 g for pituitary.

Measurement of Renin, Angiotensinogen, and Angiotensin Converting Enzyme in Plasma

The plasma concentrations of active renin, angiotensinogen, and ACE were measured as described previously.11

Statistical Analysis

Estimates of recovery are presented as mean±SD; other data are presented as mean±SEM. When more than half of the samples comprising a mean had values below the minimum detectable, the sample mean is shown as less than the minimum detectable. Data were analyzed by analysis of variance of the five groups of rats (A through E). For Ang II levels, the a priori hypothesis was that converting enzyme inhibition and nephrectomy would reduce these levels, and comparisons with control were examined by Dunnett's one-tailed test (mean<

control). For other parameters [Ang-(1-7), Ang-(1-9),

Ang I, Ang II–Ang I ratio, renin, angiotensinogen, and

ACE], comparisons with control were examined with Dunnett's two-tailed test. Other comparisons between

groups were made with the Games-Howell test. When

values were below the minimum detectable, they were

set at half the minimum detectable for statistical calcula-

tions. Logarithmic transformation of data was performed

when appropriate to obtain similar variances among

groups. Analyses were performed with SUPER-


TABLE 1. Recoveries and Minimum Detectable Levels of Angiotensin Peptides in Plasma, Blood, and Tissues
This result indicates a dominant role for ACE in the production of circulating Ang II in intact rats.

As seen for plasma, the effects of ramipril on tissue Ang II levels were dependent on the concomitant changes in Ang I levels. Ramipril caused a statistically significant fall in Ang II–Ang I ratio in all tissues except adrenal (Table 3, Fig 3), indicating a major role for ACE in Ang II generation in these tissues. For all tissues except kidney and adrenal, however, the fall in Ang II levels was less than the fall in Ang II–Ang I ratio because of the associated increase in Ang I levels; for aorta, brown adipose tissue, adrenal, and brain, the fall in Ang II levels did not achieve statistical significance (Table 3, Fig 3). Ramipril increased Ang-(1-7) levels in lung and heart and Ang-(1-9) levels in heart, but the other tissues showed no change in the levels of these peptides (Table 3).

Effect of Nephrectomy on Angiotensin Peptides in Plasma and Tissues

Nephrectomy had variable effects on Ang II and Ang I levels in plasma and tissues. For plasma, Ang II and Ang I showed little change at 24 hours after nephrectomy, but by 48 hours, Ang I levels had fallen to 7% of control, although the 60% fall in Ang II levels did not achieve statistical significance; the Ang II–Ang I ratio increased fivefold (Table 2, Fig 4A and 4B).

All tissues except brain showed a fall in Ang II levels with nephrectomy, and for most tissues Ang II levels were lower at 48 hours than at 24 hours (Table 3, Fig 4B). Whereas heart showed a significant fall in Ang I levels, for other tissues the Ang I levels were in most cases at or below the minimum detectable, and the effect of nephrectomy could not be assessed (Fig 4A). Similarly, the levels of Ang-(1-7) and Ang-(1-9) in plasma and tissues were too low for an effect of nephrectomy to be detected (Table 3).

Effect of Ramipril on Angiotensin Peptides in Plasma and Tissues of Anephric Rats

In contrast to the results obtained for intact rats, ramipril had no effect on plasma levels of Ang-(1-7), Ang II, Ang-(1-9), and Ang I in anephric rats (Table 2, Fig 4C), despite maximal suppression of plasma ACE activity (Table 4). The apparent 285% increase in
plasma Ang II levels in anephric rats given ramipril was not statistically significant (Table 2, Fig 4C) and was due to high Ang II values (29 and 67 fmol/mL) in two rats, whereas the other four rats had Ang II levels of 2.7 to 4.6 fmol/mL. Thus, it would appear that the persistent plasma Ang II levels of anephric rats are formed by a pathway independent of ACE.

Ramipril produced significant falls in Ang II levels in lung and adrenal of anephric rats (Table 3, Fig 4C). For aorta, Ang II levels fell below the minimum detectable, but this decrease failed to achieve statistical significance, because aortic Ang II levels for 48-hour nephrectomized rats were close to the minimum detectable. Moreover, the percent change for aorta shown in Fig 4C may be underestimated, because values below the minimum detectable were set at half the minimum detectable for this calculation. However, it is of note that for heart, brown adipose tissue, pituitary, and brain of 48-hour nephrectomized rats, Ang II levels were unaffected by ramipril. Ramipril had no effect on Ang I, Ang-(1-7), or Ang-(1-9) levels in tissues of anephric rats.

**Effect of Nephrectomy on Angiotensin Peptides in Blood**

The persistence of plasma Ang II levels 48 hours after nephrectomy led us to assess the validity of these measurements. One concern was the possible generation of Ang II by tissue proteases during the collection of trunk blood. We examined this possibility by studying conscious unrestrained rats with carotid arterial cannulas, and blood was collected directly into GTC/TFA. Although the basal levels of Ang II and Ang I for cannulated rats were somewhat higher than for decapitated rats, the changes after nephrectomy in blood Ang II and Ang I levels for these cannulated rats were similar to those for plasma (Table 2). Blood Ang I levels fell to 20% of control at 48 hours after nephrectomy, and Ang II showed a smaller though statistically significant fall, with a twofold increase in Ang II-Ang I ratio (Table 2).

Another concern was the possibility that a generalized activation of serine proteases caused Ang II formation after nephrectomy. To investigate this possibility we measured bradykinin levels in blood from the rats with carotid arterial cannula. Bradykinin levels in blood showed no change with nephrectomy, being 1.2±0.1 fmol/mL (mean±SEM, n=6) for control rats and 1.1±0.2 and 1.1±0.1 fmol/mL at 24 and 48 hours, respectively, after nephrectomy.

In a separate experiment, 48-hour nephrectomized rats administered ramipril were anesthetized with ether, and blood was collected from the aorta directly into GTC/TFA. These rats showed a persistence of circulating Ang II and Ang I (Table 2), and the identity of these peptides was confirmed by the radioimmunoassay of HPLC fractions with both amino-terminal- and carboxy-terminal-directed antisera (Fig 5).

**Discussion**

Through precise measurement of Ang-(1-7), Ang II, Ang-(1-9), and Ang I levels in plasma, blood, and tissues and study of the effects of nephrectomy and ACE inhibition on these levels, we obtained valuable information concerning the mechanism by which angiotensin peptides are formed in rat plasma and tissues. For control rats, the levels of Ang II in all tissues examined were much higher than could be accounted for by the plasma content of tissues and were consistent with local Ang II formation by tissues. That tissue Ang II levels were much higher than those of Ang I suggests that conversion of Ang I to Ang II is much more efficient in tissues than in plasma. This proposal is supported by the predominant tissue localization of ACE and evidence that tissues are the main site of conversion of circulating Ang I, Ang II, Ang-(1-7), Ang-(1-9), and Ang I levels in plasma, blood, and tissues and study of the effects of nephrectomy and ACE inhibition on these levels, we obtained valuable information concerning the mechanism by which angiotensin peptides are formed in rat plasma and tissues. For control rats, the levels of Ang II in all tissues examined were much higher than could be accounted for by the plasma content of tissues and were consistent with local Ang II formation by tissues. That tissue Ang II levels were much higher than those of Ang I suggests that conversion of Ang I to Ang II is much more efficient in tissues than in plasma. This proposal is supported by the predominant tissue localization of ACE and evidence that tissues are the main site of conversion of circulating Ang I, Ang II, Ang-(1-7), Ang-(1-9), and Ang I.
Table 3. Angiotensin Peptide Levels in Tissues of Control Rats, Rats Given Ramipril, Nephrectomized Rats, and Nephrectomized Rats Given Ramipril

<table>
<thead>
<tr>
<th>Group</th>
<th>Ang-(1-7)</th>
<th>Ang II</th>
<th>Ang-(1-9)</th>
<th>Ang I</th>
<th>Ang II-Ang I Ratio, fmol/fmol</th>
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<td>Kidney</td>
<td></td>
<td></td>
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<tr>
<td>A</td>
<td>31±7</td>
<td>102±13</td>
<td>64±17</td>
<td>62±10</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td>B</td>
<td>33±4</td>
<td>25±2*</td>
<td>57±13</td>
<td>46±8</td>
<td>0.6±0.1*</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>A</td>
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<td>1.5±0.3*</td>
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<td>C</td>
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<tr>
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<td>&lt;5.0</td>
<td>3.4±0.7</td>
<td>8±1*</td>
</tr>
<tr>
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<td>&lt;4.4</td>
<td>9±2†</td>
<td>&lt;5.0</td>
<td>3.7±0.9</td>
<td>3.3±0.8*</td>
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</tr>
<tr>
<td>A</td>
<td>&lt;13</td>
<td>14±2</td>
<td>15±4</td>
<td>&lt;4</td>
<td>&gt;3.5</td>
</tr>
<tr>
<td>B</td>
<td>18±5</td>
<td>9±3</td>
<td>18±4</td>
<td>9±3</td>
<td>1.3±0.3*</td>
</tr>
<tr>
<td>C</td>
<td>&lt;13</td>
<td>8±3</td>
<td>24±5</td>
<td>&lt;4</td>
<td>...</td>
</tr>
<tr>
<td>D</td>
<td>&lt;13</td>
<td>10±2</td>
<td>15±2</td>
<td>&lt;4</td>
<td>...</td>
</tr>
<tr>
<td>E</td>
<td>&lt;13</td>
<td>9±2</td>
<td>15±4</td>
<td>3±1</td>
<td>...</td>
</tr>
<tr>
<td>Pituitary</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>&lt;48</td>
<td>123±30</td>
<td>&lt;47</td>
<td>&lt;24</td>
<td>7.9±1.5</td>
</tr>
<tr>
<td>B</td>
<td>&lt;48</td>
<td>59±10‡</td>
<td>&lt;47</td>
<td>34±8</td>
<td>2.3±0.9‡</td>
</tr>
<tr>
<td>C</td>
<td>&lt;48</td>
<td>51±7‡</td>
<td>&lt;47</td>
<td>&lt;24</td>
<td>...</td>
</tr>
<tr>
<td>D</td>
<td>&lt;48</td>
<td>39±16*</td>
<td>&lt;47</td>
<td>28±11</td>
<td>...</td>
</tr>
<tr>
<td>E</td>
<td>&lt;48</td>
<td>27±6*</td>
<td>&lt;47</td>
<td>&lt;24</td>
<td>...</td>
</tr>
</tbody>
</table>

Ang indicates angiotensin. Data are mean±SEM; n=6-8, except for pituitary, where n=4. A, control rats; B, rats given ramipril (10 mg/kg); C, 24-hour nephrectomized rats; D, 48-hour nephrectomized rats; E, 48-hour nephrectomized rats given ramipril (10 mg/kg). All data have been corrected for peptide recovery and antibody cross-reactivity.

*P<.01, compared with group A.
†P<.05, compared with group D.
‡P<.05, compared with group A.
§P<.01, compared with group D.
We have previously reported the measurement of Ang-(1-7), Ang II, Ang-(1-9), and Ang I in kidney, brain, and pituitary and described the effects of ACE inhibition on angiotensin peptide levels in the kidney. Other workers have reported Ang II levels in heart and adrenal that are in agreement with the present findings. In addition, Nagano et al have reported a decrease in cardiac Ang II levels with ACE inhibition, although these authors found no effect of nephrectomy on cardiac Ang II levels, in contrast to the present findings. This discrepancy may result from the use by these authors of tissue that was frozen and thawed before analysis, because we have previously shown that freezing and thawing of tissue may result in artifactual generation of angiotensin peptides.

The considerable variation in Ang II levels between tissues may represent differences in local tissue Ang II production. Another possibility is that tissues with high Ang II receptor levels may have higher levels of Ang II peptide due to receptor occupancy by Ang II. This mechanism may account in part for the high levels of Ang II measured in adrenal, pituitary, and aorta, where Ang II receptors are abundant.

Ramiprilat, the active drug of ramipril, is relatively lipophilic and able to penetrate the blood-brain barrier and inhibit ACE activity in brain and cerebrospinal fluid. Gohlke et al have shown a rapid diffusion of [3H]ramiprilat through the rabbit thoracic aorta in vitro. Unger et al have previously shown that ramipril administration at either 1 or 10 mg/kg per day causes marked inhibition of ACE activity in plasma and tissues and reduction of blood pressure of stroke-prone spontaneously hypertensive rats. The present results are in agreement with these previous studies in that we achieved maximal suppression of plasma ACE activity, a 98% reduction in plasma Ang II–Ang I ratio, and 63% to 97% reduction in Ang II–Ang I ratio in all tissues except adrenal. Although we cannot claim that tissue ACE activity was completely inhibited, the effects of ramipril on tissue angiotensin peptides allow us to conclude that ACE plays a dominant role in Ang II formation in plasma and tissues. The failure of ramipril to cause a statistically significant suppression of either Ang II levels or Ang II–Ang I ratio in adrenal of intact rats is of interest, given that an effect was readily seen in adrenal of anephric rats, and suggests that for adrenal of intact rats, Ang II may be formed predominantly by a mechanism independent of ACE.

The present data emphasize that the effect of ACE inhibition on plasma and tissue levels of Ang II is dependent on the concomitant changes in Ang I levels. The increase in tissue Ang I levels with ramipril administration may represent either trapping of the increased plasma Ang I within the tissue or an increased production of Ang I by the tissue due to tissue uptake of kidney-derived renin. That several tissues including brown adipose tissue, adrenal, brain, and pituitary did not show an increase in Ang I levels with ramipril administration suggests that the increase in Ang I levels in lung, heart, and aorta may have resulted from a tissue-specific increase in uptake of kidney-derived renin from plasma.

We used nephrectomy to examine the role of kidney-derived renin in angiotensin peptide formation in plasma and tissues. Two qualifications need to be made concerning the results obtained from anephric rats. First, although renin was rapidly cleared from plasma, it is known to be cleared from tissues at a slower rate, and we do not know whether this process was complete by 48 hours after nephrectomy. In the present study, Ang II levels were lower at 48 hours than at 24 hours for most tissues after nephrectomy. If one accepts that the fall in tissue angiotensin peptide levels after nephrectomy was due to the loss of kidney-derived renin from the tissue, then these data indicate that this process takes longer than 24 hours.

Second, circulating angiotensinogen levels of control rats are lower than the Michaelis constant (Km) for the renin-angiotensinogen reaction, and consequently, an increase in angiotensinogen level will increase Ang I formation by a given level of renin. Therefore, the fourfold to eightfold increase in plasma angiotensinogen levels that occurred after nephrectomy may counteract the effect of lower renin levels on angiotensin peptide formation. It may also be argued that the fall in plasma and tissue Ang II levels from 24 to 48 hours after nephrectomy was due in part to the fall in plasma angiotensinogen levels that occurred at this time. Given these qualifications, the present data demonstrate a marked dependence of plasma and tissue angiotensin peptide formation on kidney-derived renin. Plasma Ang I levels fell to 7% of control, and Ang II levels in all tissues except brain showed a 59% to 78% fall after nephrectomy. This represents a minimal estimate of the role of kidney-derived renin in Ang II formation in plasma and tissues because of the likely concurrent amplification by the increased angiotensinogen levels of peptide formation by either residual kidney-derived renin or other pathways of angiotensin peptide formation. Moreover, as discussed below, Ang II formation in plasma, heart, brown adipose tissue, pituitary, and brain of anephric rats is unlikely to be due to renin. As a corollary, the present data indicate that locally synthesized renin makes little contribution to angiotensin peptide formation in tissues.

Several authors have examined the effect of nephrectomy on adrenal Ang II levels. Aguilera et al and Kim et al reported no effect of nephrectomy on adrenal

### Table 4. Renin, Angiotensinogen, and Angiotensin Converting Enzyme Levels in Plasma of Control Rats, Rats Given Ramipril, Nephrectomized Rats, and Nephrectomized Rats Given Ramipril

<table>
<thead>
<tr>
<th>Group</th>
<th>Renin, pmol/mL/h</th>
<th>Angiotensinogen, pmol/mL</th>
<th>Angiotensin Converting Enzyme, U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13.5±0.9</td>
<td>574±23</td>
<td>207±18</td>
</tr>
<tr>
<td>B</td>
<td>310±40*</td>
<td>232±15*</td>
<td>&lt;10*</td>
</tr>
<tr>
<td>C</td>
<td>0.7±0.2*</td>
<td>364±137*</td>
<td>236±18</td>
</tr>
<tr>
<td>D</td>
<td>0.2±0.1*</td>
<td>2501±231*</td>
<td>325±26*</td>
</tr>
<tr>
<td>E</td>
<td>0.4±0.1*</td>
<td>2479±296*</td>
<td>&lt;10*</td>
</tr>
</tbody>
</table>

Data are mean±SEM; n=6. A, control rats; B, rats given ramipril (10 mg/kg); C, 24-hour nephrectomized rats; D, 48-hour nephrectomized rats; E, 48-hour nephrectomized rats given ramipril (10 mg/kg).

*P<.01, compared with control (group A).
Ang II levels, although these workers studied tissue that was frozen and thawed before analysis. Other workers\(^{19,21}\) found a decrease of greater than 50%, with a persistence of adrenal Ang II levels after nephrectomy, in agreement with the present data. These data are of interest, given that nephrectomy causes a several-fold increase in adrenal renin\(^{29,30}\) and Ang II receptors,\(^{32}\) both of which may act to prevent a fall in adrenal Ang II levels after nephrectomy. The 59% fall in adrenal Ang II levels after nephrectomy measured in the present study indicates that kidney-derived renin is the major determinant of adrenal Ang II formation and suggests that adrenal renin may play little role in adrenal Ang II formation in intact rats.

Study of the effect of ramipril in anephric rats has given important information on the role of renin or reninlike enzymes in angiotensin peptide formation after nephrectomy. It is of note that plasma ACE levels increased after nephrectomy. Thus, if Ang II formation in anephric rats were via the formation of Ang I, one would expect the role of ACE (and the effect of ACE inhibition) to be at least equal to that seen in intact rats. For lung, adrenal, and aorta of anephric rats, a continuing role for ACE (and Ang I) is indicated by the suppression of Ang II levels in these tissues by ramipril. Therefore, at least for these three tissues of the anephric rat, a role for renin or reninlike enzyme in angiotensin peptide formation is indicated. This may represent residual kidney-derived renin or locally synthesized renin or reninlike enzyme. However, for plasma and the other tissues of anephric rats, ramipril was without effect on Ang II levels. This result indicates that Ang II formation in plasma, heart, brown adipose tissue, pituitary, and brain of anephric rats was independent of ACE and is therefore unlikely to involve either Ang I formation or renin or reninlike enzyme. For these tissues and plasma, Ang II may be formed directly from angiotensinogen by the action of a serine protease (Fig 1). The operation of such a pathway appears to be particularly likely in plasma, given that Ang I levels showed a much greater fall than Ang II levels after nephrectomy. As mentioned above, such a pathway may have been amplified fourfold to eightfold in anephric rats by the increase in plasma angiotensinogen levels after nephrectomy. For the 48-hour nephrectomized rats, plasma Ang II levels were 39% of control, in association with a fourfold increase in plasma angiotensinogen levels. If angiotensinogen levels had not increased fourfold, plasma Ang II levels may have fallen to 10% of control, suggesting that this alternate pathway may contribute 10% of plasma Ang II formation in intact rats.

**FIG 3.** Bar graphs show percentage change in angiotensin II (top) and ratio of angiotensin II to angiotensin I (bottom) in plasma, kidney, lung, heart, aorta, brown adipose tissue (BAT), adrenal, pituitary (Pit.), and brain after administration of ramipril (10 mg/kg) to rats 4 hours before death. Solid columns indicate statistically significant changes; open columns indicate nonsignificant changes. Calculated from data shown in Tables 2 and 3.

**FIG 4.** Bar graphs show percentage change in angiotensin I (A) and angiotensin II (B) levels in plasma, lung, heart, aorta, brown adipose tissue (BAT), adrenal, pituitary (Pit.), and brain of rats 48 hours after nephrectomy and angiotensin II levels (C) after administration of ramipril (10 mg/kg) to 48-hour nephrectomized rats 4 hours before death. Solid columns indicate statistically significant changes; open columns indicate nonsignificant changes. Calculated from data shown in Tables 2 and 3.
Our results for circulating and tissue levels of Ang II in anephric rats raise the question of whether prorenin has a role in angiotensin peptide formation. As we discussed above, at least for lung, adrenal, and aorta of anephric rats, a role for renin or reninlike enzyme in angiotensin peptide formation is indicated, consistent with a role for activated prorenin. However, there is considerable controversy concerning prorenin in the rat. Although some studies have reported prorenin levels in plasma of anephric rats, it has been suggested that the kidney is the primary source of circulating prorenin. For tissues such as adrenal, where renin synthesis occurs, activated prorenin may play a role, although as discussed above the present data demonstrate that kidney-derived renin is the predominant determinant of Ang II formation in the adrenal.

Our finding of persistent Ang II levels in plasma of anephric rats is supported by previous studies in the rat but is contrary to our previous results for anephric humans, in which plasma levels of Ang II and Ang I were very low. Moreover, other studies in the rat have shown plasma Ang II levels to fall to undetectable levels after nephrectomy. The reason for these discrepancies is unknown. In the present study, we took particular care to confirm that the angiotensin peptides identified were authentic and not the result of artifactual generation during sample processing. The difference between the present data and our previous results for anephric humans may be due in part to the greater increase in plasma angiotensinogen levels, being four- to eightfold in the anephric rat compared with only a twofold increase in anephric humans.

In conclusion, we have shown that tissue levels of Ang II are higher than can be accounted for by trapping of plasma and are consistent with local tissue production of angiotensin peptides. We have demonstrated a major role for both kidney-derived renin and ACE in this local tissue production of Ang II. In addition, we have demonstrated that alternate pathways, independent of kidney-derived renin and ACE, may also contribute to circulating and tissue levels of Ang II.

Acknowledgments

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Nephrectomy, converting enzyme inhibition, and angiotensin peptides.
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