Intrarenal De Novo Production of Angiotensin I
in Subjects With Renal Artery Stenosis

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To estimate the renal extraction and de novo production of angiotensin I and to assess the contribution of blood-borne renin to renal angiotensin I production, the aortic and renal venous plasma levels of renin and intact [125I]angiotensin I and endogenous angiotensin I during continuous systemic intravenous infusion of monoiodinated [125I]angiotensin I were measured in subjects with unilateral renal artery stenosis (n=8) who were treated with captopril (50 mg b.i.d.). Results demonstrated that 80% of angiotensin I delivered by the renal artery was extracted both by the affected and the unaffected kidney and that on both sides a major part of angiotensin I in the renal vein was derived from intrarenal de novo production. Production of plasma angiotensin I was in excess over extraction (p<0.01) on the affected side, whereas extraction was in excess over production (p<0.01) on the contralateral side. The plasma level of de novo intrarenally produced angiotensin I in the renal vein was seven times higher on the affected side than the contralateral side. This difference was by far too big to be explained by a difference in the transit time of blood between the two kidneys, by an augmented production of angiotensin I in the circulating blood passing through the affected kidney due to the higher level of venous plasma renin activity in that kidney, or by the combination of both. We conclude that intrarenal production of angiotensin I in a compartment outside the circulating blood is a major source of this peptide in the renal vein, and that in kidneys with artery stenosis in situ, synthesized renin is an important component of this source. To the extent that local angiotensin I production by intrarenally synthesized renin is a sine qua non for a local physiological role of renin, our results support the hypothesis that renin may have such a role. (Hypertension 1990;16:555–563)

Angiotensin II (Ang II) in the kidney subserves important functions in the control of renal blood flow, glomerular filtration rate, and tubular sodium handling. It does so by acting on specific receptors located on blood vessels and mesangial and tubular cells.1–3 These receptors are stimulated by Ang II delivered from the systemic circulation by the renal artery and most likely also by Ang II formed within the kidney. Intrarenally formed Ang II in turn originates from conversion of arterially delivered angiotensin I (Ang I) and possibly also from locally produced Ang I. Intrarenally produced Ang I may also arise from two sources, namely arterially delivered renin or in situ synthesized renin. The interactions between systemically delivered and locally produced components of the renin-angiotensin system in the kidney are not well understood. Detailed information on how much and where Ang I and Ang II are produced in the kidney would help to answer this question.

In a previous study in subjects with essential hypertension, we demonstrated that the levels of radio-labeled Ang I in arterial and venous plasma during systemic intravenous infusion of purified monoiodinated [125I]Ang I can be used for calculating the Ang I extraction rate in different vascular beds, including the kidney, at least under conditions of angiotensin converting enzyme (ACE) inhibition.4 Circulating Ang I is rapidly metabolized by the tissues, and this process includes degradation into inactive peptides as well as conversion to Ang II. By combining data on regional [125I]Ang I extraction with results of measurements of endogenous Ang I, information can be obtained on regional Ang I production.

The present study was aimed at collecting quantitative data on intrarenal Ang I production in subjects...
with renal artery stenosis. For this purpose \[^{[25]}\text{I}\] \text{Ang I} was administered systemically, which enabled us to collect information on Ang I metabolism and production in the kidney affected by renal artery stenosis as well as in the contralateral unaffected kidney.

**Methods**

**Chemicals**

\[^{[Ile^5]}\text{Ang-(1-10) decapeptide (Ang I), [Ile^5]}\text{Ang-(1-8) octapeptide (Ang II), [Ile^5]}\text{Ang-(2-8) heptapeptide (Ang III), and [Ile^5]}\text{Ang-(1-7) were obtained from Bachem, Dielsdorf, Switzerland.}\[^{[Ile^5]}\text{Ang-(2-10) nonapeptide was from Senn Chemicals, Dielsdorf, Switzerland.}\[^{[Ile^5]}\text{Ang-(3-8) hexapeptide, [Ile^5]}\text{Ang-(4-8) pentapeptide, and}\[^{[Ile^5]}\text{Ang-(1-4) tetrapeptide were from Peninsula Laboratories, Belmont, Calif. The World Health Organization standards of Ang I and Ang II (85/536 and 86/538) were from the National Institute for Biological Standards and Control, London, UK. Methanol and ort/o-phosphoric acid (both analytical grade) were from Merck, Darmstadt, FRG. Bovine serum albumin (BSA) was from Sigma Chemical Co., St. Louis, Mo. Water for high-performance liquid chromatography (HPLC) was prepared with a Milli-Q system from Waters Chromatography Division, Milipore Corp., Milford, Mass. The statine-containing lyl-silica (Sep-Pak C18, Waters) and ort/u?-phosphoric acid (both analytical grade) were from Sigma Chemical Co., St. Louis, Mo. Water for high-performance liquid chromatography (HPLC) was prepared with a Milli-Q system from Waters Chromatography Division, Milipore Corp., Milford, Mass. The statine-containing renin inhibitor CGP 29,287 was a kind gift of Dr. K. Hofbauer (Ciba-Geigy, Basel, Switzerland).**

**Blood Sampling**

Blood for angiotensins measurements was rapidly (7–13 seconds) drawn by a plastic syringe containing the following inhibitors (0.5 ml inhibitor solution in 10 ml blood): 6.25 mM disodium EDTA, 1.25 mM, 1,10-phenantroline, and 100 nM of the renin inhibitor CGP 29,287 (final concentrations in blood) and was then transferred into prechilled polystyrene tubes. The blood samples were centrifuged at 3,000 g for 10 minutes at 4° C. Plasma was stored at −70° C, extracted within 2 days and assayed within 2 weeks.

Blood for plasma renin activity (PRA) measurements was collected in polystyrene tubes containing EDTA (0.2 ml disodium EDTA in 10 ml blood, final concentration of 6.25 mM). The samples were centrifuged at 3,000 g for 10 minutes at room temperature and plasma was stored at −20° C.

**Separation of Angiotensins by High-Performance Liquid Chromatography**

Angiotensins and their metabolites were extracted from plasma by reversible adsorption to octadecylsil-y-silica (Sep-Pak C18, Waters) and separated by reversed-phase HPLC according to the method of Nussberger et al with some modifications as described by Admiraal et al.4 Separations were performed on a reversed-phase Nucleosil C18 steel column of 250×4.6 mm and 10 μm particle size (Alltech, Eke, Belgium). Mobile phase A was 0.085% ortho-phosphoric acid containing 0.02% sodium azide. Mobile phase B was methanol. The flow was 1 ml/min and the working temperature 45° C. Extracts of 2 ml plasma were dissolved in 100 μl HPLC solvents and injected. Elution was performed as follows: 65% A/35% B (vol/vol) from 0 to 9 minutes followed by a linear gradient to 45% A/55% B until 18 minutes. The eluate was collected in 0.5-minute fractions into polystyrene tubes coated with BSA. The concentrations of \[^{[25]}\text{I}\] \text{Ang I} and \[^{[25]}\text{I}\] \text{Ang II} and their metabolites in the HPLC fractions were measured in the gamma counter. The fractions containing Ang I and Ang II were neutralized with 0.5 M sodium hydroxide and vacuum dried at 4° C.

**Assays of Angiotensins, Renin, and Renin Substrate**

The concentrations of Ang I and Ang II in the HPLC fractions were measured by radioimmunoassay. The Ang I antiserum cross-reacted with Ang-(2-10) nonapeptide (100%) but not (less than 0.1%) with Ang II, Ang III, Ang-(3-8) hexapeptide, Ang-(4-8) pentapeptide, and Ang-(1-4) tetrapeptide. The Ang II antiserum cross-reacted with Ang III (55%), Ang-(3-8) hexapeptide (73%), and Ang-(4-8) pentapeptide (100%) but virtually not (less than 0.2%) with Ang I, Ang-(2-10) nonapeptide, Ang-(1-7) heptapeptide, or Ang-(1-4) tetrapeptide.

The plasma levels of enzymatically active renin and PRA were determined by the enzyme-kinetic method, in which generated Ang I was measured by radioimmunoassay.6 In the assay of plasma renin concentration, Ang I was generated by incubation with sheep renin substrate and in the assay of PRA by incubation with endogenous substrate. Both incubations were carried out at pH 7.4 and 37° C. Renin substrate was determined as the maximal quantity of Ang I generated during incubation of plasma with an excess of purified human kidney renin.6

**Estimations of Renal Plasma Flow and Split Renal Function**

The clearance of \[^{[3]}\text{I}\] \text{iodohippurate} was determined as a measure of total effective renal plasma flow (right plus left kidney). The single kidney extraction ratio of \[^{[3]}\text{I}\] \text{iodohippurate} was also measured.

The single kidney uptake of \[^{[99]}\text{Tc}]\text{diethylenetriamine pentaacetic acid (}\[^{[99]}\text{Tc}]\text{DTPA)}\] was determined by scintillation camera renography.7 The increase in radioactivity over the kidney region during the second minute after intravenous injection of \[^{[99]}\text{Tc}]\text{DTPA} was expressed as activity ratio, that is, right/(right+left) ratio. This ratio is a measure of the single kidney contribution to the total glomerular filtration rate.

**Subjects**

Subjects with hypertension received an intravenous infusion of \[^{[15]}\text{I}\] \text{Ang I} at the time they were undergoing renal vein sampling followed by renal angiography for diagnostic purposes. Here we report the results obtained in eight subjects (six men, two
women, 22–70 years old), who proved to have unilateral renal artery stenosis (60–90% stenosis) on angiographic examination. The results in these subjects were compared with those obtained in a group of seven subjects with essential hypertension and a normal angiogram. The latter group was described in a previous paper.4 The subjects were studied while they were treated with the ACE inhibitor captopril (50 mg orally, b.i.d.). The infusions were given 4–6 hours after administration of the morning dose of captopril. The subjects had given their informed consent and the study was approved by the Hospital Ethical Review Committee.

### Infusion Protocol

Highly purified monoiodinated \([{}^{125}\text{I}]\text{Ang I}\) was prepared as described previously.4 After insertion of the catheters into the abdominal aorta and the inferior caval vein via the femoral artery and vein by the Seldinger technique, \([{}^{125}\text{I}]\text{Ang I}\) was infused via an antecubital vein at a constant rate of approximately 3 × 10^6 cpm/min (approximately 1 pmol/min) for 20 minutes. Blood samples were taken from the right and left renal vein between 10 and 20 minutes after the infusion was started. An aortic sample was taken simultaneously with each renal vein sample. The arterial and venous plasma levels of \([{}^{125}\text{I}]\text{Ang I}\) and \([{}^{125}\text{I}]\text{Ang II}\) remained constant during this period (coefficient of variation less than 4%).

### Calculations and Statistics

The renal extraction ratio (ER) of Ang I was calculated as follows:

\[
\text{ER of Ang I} = 1 - \left( \frac{[{}^{125}\text{I}]\text{Ang I}_{\text{ven}}}{[{}^{125}\text{I}]\text{Ang I}_{\text{art}}} \right)
\]

in which ven is renal venous and art is aortic. The renal venous plasma level of Ang I (\(\text{Ang I}_{\text{ven}}\)) derived from renal de novo production was calculated as follows:

\[
\text{Ang I}_{\text{ven}} = \frac{(1 - \text{ER}) \times \text{Ang I}_{\text{art}}}{\text{Ang I}_{\text{ven}} - \text{Ang I}_{\text{art}}}
\]

in which \(\text{ER}\) is extraction ratio of Ang I.

Results of measurements of angiotensins and renin were not normally distributed and are presented as medians and ranges. The Wilcoxon rank sum test was used to compare angiotensin and renin levels between groups. For calculating PRA, the levels of renin and renin substrate and the values of \(V_{\text{max}}=1.6\ \mu\text{mol}/\text{l}\) and \(K_m=1.2\ \mu\text{mol}/\text{l}\) were used to calculate Vmax.

### Results

#### Identification of Angiotensins and Their Metabolites in Plasma by High-Performance Liquid Chromatography

With the standard elution program, the radiolabeled peptides in the Sep-Pak extracts of aortic and renal venous plasma from subjects to whom \([{}^{125}\text{I}]\text{Ang I}\) was given with constant intravenous infusion were separated by HPLC into four major peaks (peaks I to IV, Figure 1). Material in these peaks was quantitatively bound to Sep-Pak and was completely recovered by elution with methanol. Peaks II, III, and IV with a retention time of 10.0, 13.5, and 16.5 minutes were identified as \([{}^{125}\text{I}]\text{Ang-(1–8)} = [{}^{125}\text{I}]\text{Ang II}, [{}^{125}\text{I}]\text{Ang-(2–10)},\) and \([{}^{125}\text{I}]\text{Ang-(1–10)} = [{}^{125}\text{I}]\text{Ang I},\) respectively. The identification was not only based on retention time but also on reactivity with the Ang I and Ang II antiserum. The Ang I antiserum, which reacted with Ang I and Ang-(2–10) but virtually not with Ang II, bound 90–100% of the radioactivity in peaks III and IV and bound less than 3% of the radioactivity in peak II. In contrast, the Ang II antiserum bound more than 90% of the radioactivity of peak II and less than 5% of the radioactivity of peaks III and IV.

The retention times of peaks II and IV were sufficiently different from each other and from the retention times of \([{}^{125}\text{I}]\text{Ang-(2–8)} = [{}^{125}\text{I}]\text{Ang III}, [{}^{125}\text{I}]\text{Ang-(3–8)}, [{}^{125}\text{I}]\text{Ang-(4–8)}, [{}^{125}\text{I}]\text{Ang-(1–7)}, [{}^{125}\text{I}]\text{Ang-(1–4)}, and [{}^{125}\text{I}]\text{tyrosine} to obtain satisfactory separations. By removing cross-reacting angiotensin peptides, the HPLC separations allowed us to perform specific measurements of unlabeled Ang I and Ang II by radioimmunoassay.

By using lower starting concentrations of methanol in the mobile phase, it was possible to separate peak I into two to three peaks (Figure 1, lower panels). One of these, peak I-B, accounting for 30–50% of the total radioactivity of peak I, had a retention time identical to that of \([{}^{125}\text{I}]\text{Ang-(1–7)}\). On the basis of its chromatographic behavior, this peak could be differentiated from all the other angiotensin peptides we tested, but the conclusion that this peak is indeed \([{}^{125}\text{I}]\text{Ang-(1–7)}\) needs further confirmation. A second subfraction, peak I-A, could be separated from peak I. This subfraction had the retention time of \([{}^{125}\text{I}]\text{tyrosine}\) and could also be differentiated from the other angiotensin metabolites we tested.

#### Split Renal Function and Renal Venous Plasma Levels of Renin and Angiotensins

The total (right plus left kidney) glomerular filtration rate, as indicated by the serum level of creatinine, was reduced in the subjects with renal artery stenosis as compared with essential hypertension (Table 1). The total effective renal plasma flow was also reduced. The presence of unilateral renal artery stenosis was reflected by differences between the two kidneys in \([{}^{99}\text{Tc}]\text{DPTA uptake}\) and \([{}^{131}\text{I}]\text{iodohippurate elimination}\). As judged from \([{}^{99}\text{Tc}]\text{DTPA uptake}\), the contribution of the kidney affected by renal artery stenosis to the total glomerular filtration rate was approximately 20%.

As expected, the aortic plasma levels of renin were higher in the subjects with renal artery stenosis than in those with essential hypertension (Table 2). Renin substrate concentration was lower in the renal artery stenosis group than in the essential hypertension group. For calculating PRA, the levels of renin and renin substrate and the values of \(K_m=1.2\ \mu\text{mol}/\text{l}\) and \(V_{\text{max}}=1.6\ \mu\text{mol Ang I/ min per milliunit renin were}
entered into the Michaelis-Menten equation. Results of this calculation were close to the actually measured values of PRA (Table 2), which indicates that the incubate of the PRA assay was free of substances interfering with the enzymatic activity of renin. Data on the renal vein PRA levels in the individual subjects with renal artery stenosis are given in Table 3.

In the renal artery stenosis group, the renal vein-to-aorta ratio of PRA was 1.92 (median) on the affected side, which was significantly \( p < 0.01 \) higher than 1.00 (Figure 2). Contralaterally this ratio was 1.05, which was not significantly different from 1.00. Similar results were obtained for renin. Thus, there was a net secretion of renin into the circulation on the affected side but not on the contralateral side. In

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**FIGURE 1.** Line graphs showing high-performance liquid chromatography of iodine-125-labeled angiotensins (Ang) in aortic and renal venous plasma on affected side of subject with renal artery stenosis who received a constant intravenous infusion of \([^{125}\text{I}]\text{Ang I}\). Peak I (upper panels) was rechromatographed using a different elution program (lower panels). Stationary phase, Nucleosil C18, 10 \( \mu \text{m} \), 250 \( \times \) 4.6 mm. Mobile phase A, ortho-phosphoric acid 0.085% (containing 0.02% sodium azide). Mobile phase B, methanol. Detection by gamma counting. Peaks II, III, and IV (upper panels) with retention times of 10.0, 13.5, and 16.5 minutes correspond with \([^{125}\text{I}]\text{Ang-(1-8)}=^{[^{125}\text{I}]\text{Ang II}}, \ [^{125}\text{I}]\text{Ang-(2-10)}, \text{ and } \ [^{125}\text{I}]\text{Ang-(1-10)}=^{[^{125}\text{I}]\text{Ang I}}, \text{ respectively. Retention times of peaks I-A and I-B (lower panels) correspond with those of } \ [^{125}\text{I}]\text{tyrosine and } \ [^{125}\text{I}]\text{Ang-(1-7)}, \text{ respectively. Arrows indicate retention times of standard radiolabeled angiotensin peptides, for instance, } 1-8=^{[^{125}\text{I}]\text{Ang-(1-8)}} \text{ and } \text{tyrosine}=^{[^{125}\text{I}]\text{tyrosine}}.\)
essential hypertension, the renal vein-to-aorta ratio of PRA is known to be higher than 1.00 and is approximately 1.25.8 The low renin ratio of the contralateral kidney in subjects with renal artery stenosis is likely to reflect the suppressed production of renin by that kidney.

The aortic plasma levels of Ang I and Ang II were higher in the subjects with renal artery stenosis than in those with essential hypertension (Table 2). As expected in subjects treated with ACE inhibitor, the plasma levels of Ang II were low, particularly in relation to the high levels of Ang I. Data on the renal venous plasma levels of Ang I and Ang II in the individual subjects with renal artery stenosis are given in Table 3.

In the renal artery stenosis group, the renal vein-to-aorta concentration ratio of Ang I was 1.97 on the affected side and 0.67 contralaterally (Figure 2). On the affected side, the ratio was higher than 1.00 ($p<0.01$). Contralaterally it was lower than 1.00 ($p<0.01$), indicating net extraction of Ang I from the systemic circulation. This contrasts with essential hypertension, where the renal vein-to-aorta ratio of Ang I on both sides is not significantly different from 1.00. The renal vein-to-aorta ratio of Ang II in the subjects with renal artery stenosis was less than 1.00 ($p<0.01$) both on the affected side and contralaterally. Thus, there was net extraction of Ang II from the circulation by both the affected and unaffected kidney.

The presence of Ang-(2-8)=Ang III in plasma has been reported.9-11 When $^{[125]}$IAng III was added to plasma before extraction, it was completely recovered in the Sep-Pak extract. However, without the addition of $^{[125]}$IAng III, very little of this radioactive metabolite was found in the plasma of our subjects.

### Table 2. Aortic Plasma Levels of Renin, Renin Substrate, Plasma Renin Activity, and Angiotensins in Subjects With Renal Artery Stenosis as Compared With Essential Hypertension

<table>
<thead>
<tr>
<th>Variables</th>
<th>Essential hypertension ($n=7$)</th>
<th>Renal artery stenosis ($n=8$)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin (milliunits/l)</td>
<td>22 (6.2-117)</td>
<td>272 (28-463)</td>
<td></td>
</tr>
<tr>
<td>Renin substrate ($\mu$mol/l)</td>
<td>1.11 (0.84-1.33)</td>
<td>0.71 (0.41-1.17)*</td>
<td></td>
</tr>
<tr>
<td>PRA calculated† ($pmol \cdot l^{-1} \cdot min^{-1}$)</td>
<td>21 (6.1-101)</td>
<td>126 (32-272)#</td>
<td></td>
</tr>
<tr>
<td>PRA measured ($pmol \cdot l^{-1} \cdot min^{-1}$)</td>
<td>25 (7.3-117)</td>
<td>128 (35-430)$\dagger$</td>
<td></td>
</tr>
<tr>
<td>Angiotensin I ($pmol/l$)</td>
<td>46 (8.9-142)</td>
<td>187 (55-308)$\S$</td>
<td></td>
</tr>
<tr>
<td>Angiotensin II ($pmol/l$)</td>
<td>2.2 (0.8-5.0)</td>
<td>10.6 (2.7-73)$\S$</td>
<td></td>
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</tbody>
</table>

Data are medians and ranges. The subjects with essential hypertension were described in a previous paper and data on plasma renin activity (PRA) and angiotensins I (Ang I) and II are from that paper.4 $^{\S}$p<0.05; $^{\D}$p<0.01 for difference from essential hypertension. $^{\dagger}K_m=1.2 \ \mu$mol/l and $V_{max}=1.6$ pmol Ang I/min per milliunit of renin were entered into the Michaelis-Menten equation to calculate PRA.
Production of Angiotensin I

The mean transit time of plasma has been reported to be 4–10 seconds (0.07–0.17 minutes) for kidneys of hypertensive patients without renal artery stenosis as well as for kidneys with a degree of renal artery stenosis similar to that in our subjects. In our subjects with renal artery stenosis, the minimum time required for circulating PRA to maintain the renal vein level of Ang I was much longer; it was 1.10 (0.48–2.01) minutes (median and range) on the affected side and 0.39 (0.01–0.84) minutes on the contralateral side. To estimate the renal vein level of Ang I derived from intrarenal production by circulating PRA, we used a transit time of 0.17 minutes and multiplied this value with the measured renal venous level of PRA. The results are presented in Figure 3. It appeared that on the affected side most of the intrarenally produced Ang I in renal venous plasma could not be accounted for by circulating PRA. On the unaffected side about half of Ang I in the renal vein could be accounted for by circulating PRA. This might indicate that the transit time we used to calculate the intrarenal Ang I production by circulating PRA was too high. An alternative explanation might be that part of Ang I produced by circulating PRA is extracted before it can reach the renal vein.

Table 3. Levels of Plasma Renin Activity and Angiotensins in Subjects With Renal Artery Stenosis

<table>
<thead>
<tr>
<th>Subject</th>
<th>Artery</th>
<th>Vein affected kidney</th>
<th>Vein unaffected kidney</th>
<th>Artery</th>
<th>Vein affected kidney</th>
<th>Vein unaffected kidney</th>
<th>Artery</th>
<th>Vein affected kidney</th>
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<td>1</td>
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<td>495</td>
<td>49</td>
<td>39.8</td>
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<td>2.8</td>
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<td>292</td>
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<td>6</td>
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<td>7</td>
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<tr>
<td>Median</td>
<td>187</td>
<td>370</td>
<td>73</td>
<td>10.6</td>
<td>4.6</td>
<td>2.8</td>
<td>128</td>
<td>301</td>
<td>134</td>
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</table>

PRA, plasma renin activity.

Figure 2. Scatterplots showing renal vein-to-aorta ratios (V/A) of plasma renin concentration, plasma renin activity (PRA), and plasma concentrations of radiolabeled and endogenous angiotensins in subjects with renal artery stenosis.
Renal Angiotensin I–II Conversion

Despite ACE inhibitor treatment, some \([^{125}\text{I}]\text{Ang I}\) and \([^{125}\text{I}]\text{Ang II}\) conversion occurred in our subjects (Table 4), who were studied 4–6 hours after oral dosing of 50 mg captopril. At that time part of plasma ACE activity is already restored. Assuming the degradation rates for \([^{125}\text{I}]\text{Ang I}\) and \([^{125}\text{I}]\text{Ang II}\) to be equal, the results of this study, showing similar renal vein-to-aorta ratios of \([^{125}\text{I}]\text{Ang I}\) and \([^{125}\text{I}]\text{Ang II}\) (Figure 2) would suggest that little \([^{125}\text{I}]\text{Ang I}\)–II conversion had occurred in the kidneys. However, no firm conclusion on Ang I–II conversion can be drawn from our results because we did not study Ang II metabolism. Moreover, while the in vivo degradation rates of \([^{125}\text{I}]\text{Ang I}\) and unlabeled Ang I show little or no difference, it is not known whether the conversion rates of the two peptides are also not different.

Discussion

Measurements of immunoreactive Ang I–like material in renal venous and arterial plasma reported by others provide evidence for net release of Ang I into the systemic circulation by kidneys affected by renal artery stenosis but not by kidneys with normal arteries. Measurements in dogs of the arterial and renal venous plasma levels of true Ang I (separated from cross-reacting immunoreactive material) demonstrated net release of this peptide into the systemic circulation when renal perfusion pressure was reduced by clamping the renal artery. Our study extends these data by measurement of the renal extraction of arterially delivered radiolabeled Ang I, which enabled us to collect information on the de novo production of Ang I in the kidneys. In a previous study, we validated the use of systemic intravenous infusion of monoiodinated \([^{125}\text{I}]\text{Ang I}\) for measuring the extraction of arterially delivered Ang I by different vascular beds, including the kidney, in subjects given captopril. In the present study, which concerned subjects with unilateral renal artery stenosis, the renal extraction ratio of Ang I appeared independent of renal plasma flow; it was about 80% both on the affected side and contralaterally, and this is not different from what we found in essential hypertension.

### Table 4. Levels of Radiolabeled Angiotensins in Subjects With Renal Artery Stenosis

<table>
<thead>
<tr>
<th>Subject</th>
<th>([^{125}\text{I}]\text{Angiotensin I (cpm/1} \times 10^3)</th>
<th>([^{125}\text{I}]\text{Angiotensin II (cpm/1} \times 10^3)</th>
<th>([^{125}\text{I}]\text{Angiotensin I infusion rate (cpm/min/1} \times 10^6)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Artery affected kidney</td>
<td>Vein affected kidney</td>
<td>Artery unaffected kidney</td>
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<tr>
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<td>1,119</td>
<td>173</td>
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<tr>
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<td>Median</td>
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<td>125</td>
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**Renal Angiotensin I–II Conversion**

Despite ACE inhibitor treatment, some \([^{125}\text{I}]\text{Ang I}\) and \([^{125}\text{I}]\text{Ang II}\) conversion occurred in our subjects (Table 4), who were studied 4–6 hours after oral dosing of 50 mg captopril. At that time part of plasma ACE activity is already restored. Assuming the degradation rates for \([^{125}\text{I}]\text{Ang I}\) and \([^{125}\text{I}]\text{Ang II}\) to be equal, the results of this study, showing similar renal vein-to-aorta ratios of \([^{125}\text{I}]\text{Ang I}\) and \([^{125}\text{I}]\text{Ang II}\) (Figure 2) would suggest that little \([^{125}\text{I}]\text{Ang I}\)–II conversion had occurred in the kidneys. However, no firm conclusion on Ang I–II conversion can be drawn from our results because we did not study Ang II metabolism. Moreover, while the in vivo degradation rates of \([^{125}\text{I}]\text{Ang I}\) and unlabeled Ang I show little or no difference, it is not known whether the conversion rates of the two peptides are also not different.

**Discussion**

Measurements of immunoreactive Ang I–like material in renal venous and arterial plasma reported by others provide evidence for net release of Ang I into the systemic circulation by kidneys affected by renal artery stenosis but not by kidneys with normal arteries. Measurements in dogs of the arterial and renal venous plasma levels of true Ang I (separated from cross-reacting immunoreactive material) demonstrated net release of this peptide into the systemic circulation when renal perfusion pressure was reduced by clamping the renal artery. Our study extends these data by measurement of the renal extraction of arterially delivered radiolabeled Ang I, which enabled us to collect information on the de novo production of Ang I in the kidney. In a previous study, we validated the use of systemic intravenous infusion of monoiodinated \([^{125}\text{I}]\text{Ang I}\) for measuring the extraction of arterially delivered Ang I by different vascular beds, including the kidney, in subjects given captopril. In the present study, which concerned subjects with unilateral renal artery stenosis, the renal extraction ratio of Ang I appeared independent of renal plasma flow; it was about 80% both on the affected side and contralaterally, and this is not different from what we found in essential hypertension.
From the renal extraction ratio of Ang I and from the measured levels of intact endogenous Ang I in the aorta and renal vein, it could be calculated that in the subjects with renal artery stenosis 70–90% of Ang I in the renal vein was derived from intrarenal production and not from delivery via the renal artery, both on the affected side and on the contralateral side. Again, this is similar to what we found in essential hypertension. The level of intrarenally produced Ang I in the renal vein in the subjects with renal artery stenosis was seven times higher on the affected side than on the contralateral side and 10 times higher than in essential hypertension. It has been reported that in hypertensive subjects the plasma flow per unit weight of kidneys with a degree of artery stenosis similar to that in our subjects is no less than half of the flow of kidneys with normal arteries. Thus, the observed differences in the levels of intrarenally produced Ang I in the renal vein were likely to be due, at least in part, to differences in the production rate of this peptide.

One assumption is that these differences were caused by the higher levels of renin in the renal circulation in subjects with renal artery stenosis as compared with those with essential hypertension. By taking into account the measured levels of PRA and the reported values of the mean transit time of blood for kidneys with and without artery stenosis, it was possible to estimate the contribution of circulating PRA to the intrarenal production of Ang I. We took the highest value of the renal transit time of blood (0.17 minute) reported in the literature to avoid underestimation of the contribution of circulating PRA. It appeared that in our subjects with renal artery stenosis less than 10–20% of intrarenally produced Ang I on the affected side could be accounted for by circulating PRA. Also, in essential hypertension only a minor part, less than 20–30%, of intrarenally produced Ang I can be accounted for by circulating PRA. These figures are indeed maximum values, not only because the transit time values we chose were on the high side but also because not all intrarenally produced Ang I may have reached the renal vein.

Locally synthesized renin contributes to the level of PRA in the renal circulation and thereby to the production of Ang I in plasma during its passage through the kidneys. From the data above, however, we have to conclude that most Ang I in the renal vein is not derived from the action of circulating renin on circulating renin substrate but is produced in a compartment outside the circulating blood. Ang I in this compartment is likely to be produced by in situ synthesized renin because the renal venous level of Ang I derived from this compartment, in our subjects with renal artery stenosis, was higher on the affected side than on the contralateral side, a difference that was much too big to be explained solely by a difference in the renal transit time of blood.

The anatomic localization of this compartment is unknown, but our data suggest two possibilities. One possibility is the vascular wall. Multiple lines of evidence support the existence of a vascular renin-angiotensin system. Vascular renin appears to originate from binding or uptake of plasma renin or prorenin, but there is also evidence for synthesis of renin in situ. Binding of plasma renin to vascular tissue may result in a high local concentration of this enzyme and a high local production rate of angiotensins. Vascular renin in the kidney may originate from renin that is delivered to the renal circulation via the renal artery as well as from renin that is locally secreted into the renal circulation.

Alternatively, Ang I may be formed by renin that is secreted by the juxtaglomerular cells into the renal interstitium. Renal lymph contains renin in high concentrations and interstitial renin is thought to enter the blood compartment at the level of the peritubular capillaries. These sites may therefore represent the compartment from which most of Ang I in the renal vein is derived.

The results of this study seem to indicate that in our subjects who were receiving ACE inhibitor treatment little Ang I–II conversion occurred in the kidneys. However, as explained under Results, our data on Ang I–II conversion are incomplete. In untreated dogs receiving Ang I via infusion into the renal artery, about 20% of radioactivity in the renal venous plasma of the infused kidney was recovered in the form of Ang II. Intrarenally infused unlabeled Ang I in these animals was also partly converted to Ang II, and the formed Ang II had significant effects on renal hemodynamics. Ang II is not only present in the blood compartment of the kidney; this peptide has also been detected in renal lymph. The levels that were measured in renal lymph were higher as compared with plasma, but there is some doubt on these measurements because of the possibility that part of Ang II was formed during the procedure of lymph collection.

To the extent that local Ang I production by intrarenally synthesized renin is a sine qua non for a local physiological role of renin, the results of our study support the hypothesis that renin may have such a role.

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References


KEY WORDS • angiotensin I • renin • renal artery stenosis
Intrarenal de novo production of angiotensin I in subjects with renal artery stenosis.
P J Admiraal, F H Derkx, A H Danser, H Pieterman and M A Schalekamp

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