Regional Angiotensin II Production in Essential Hypertension and Renal Artery Stenosis


To study regional metabolism and production of angiotensin II, we measured steady-state plasma levels of $^{125}$I-angiotensin I and II and endogenous angiotensin I and II in the aorta and the antecubital, femoral, renal, and hepatic veins during systemic infusion of $^{125}$I-angiotensin I or II. Extraction of arterially delivered angiotensin II ranged from 30–50% in the limbs to 80–100% in the renal and hepatomesenteric vascular beds both in essential hypertension ($n=13$) and in unilateral renal artery stenosis ($n=7$). Across the limbs, 20–30% of arterially delivered angiotensin I was converted to angiotensin II in both groups, and there was no arteriovenous gradient in endogenous angiotensin II. No conversion of arterially delivered angiotensin I was detected across the renal and hepatomesenteric beds, and there was net extraction of angiotensin II from the systemic circulation by these beds. Although regional production of angiotensin I at tissue sites made a significant contribution to its level in the veins, little of this locally produced angiotensin I reached the regional veins in the form of angiotensin II, even in the kidney with artery stenosis, where the venous levels of locally produced angiotensin I were particularly high. These results provide no evidence for a source of circulating angiotensin II other than blood-borne angiotensin I and illustrate the high degree of compartmentalization of angiotensin I and II production. (Hypertension 1993;21:173-184)
Organization Ang I and Ang II standards (86/536 and 86/538) were from the National Institute for Biological Standards and Control, London. Methanol and orthophosphoric acid (both analytical grade) were from E. Merck, Darmstadt, FRG. Bovine serum albumin and ACE from rabbit lung were from Sigma Chemical Co., St. Louis, Mo. Water for high-performance liquid chromatography was prepared with a Milli-Q system from Waters Chromatography Division, Milford, Mass. The statine-containing renin inhibitor CGP 29,287 was a gift of Dr. K. Hofbauer (CIBA-GEIGY, Basel, Switzerland). Human kidney ACE was a gift of Dr. F. Alhenc-Gelas and Dr. P. Corvol (Institute National de la Santé et de la Recherche Médicale, Paris). The ACE inhibitor lisinopril was a gift of Dr. J. Bouman (Merek Sharp & Dohme, Haarlem, The Netherlands).

**Blood Sampling**

Blood for angiotensin measurements was rapidly drawn (within approximately 10 seconds from the aorta and the antecubital and femoral veins, and within approximately 15 seconds from the renal veins) with a plastic 10-ml syringe containing 0.5 ml inhibitor solution (6.25 mM disodium EDTA, 1.25 mM 1,10-phenanthroline, and 100 nM CGP 29,287, final concentrations in blood) and was transferred into prechilled plastic tubes. The blood samples were centrifuged at 3,000g for 10 minutes at 4°C. Plasma was stored at −20°C and was extracted within 2 days.

Blood for plasma renin activity (PRA) measurements was collected into 10-ml plastic tubes containing 0.2 ml sodium heparin (final concentration, 200 USP units in 10 ml blood). Samples were centrifuged at 3,000g for 10 minutes at room temperature, and plasma was stored at −70°C.

Blood for in vitro Ang I-to-II conversion studies was collected from the antecubital vein of healthy volunteers into tubes containing 0.04 ml sodium heparin (final concentration, 200 USP units in 10 ml blood). Samples were centrifuged at 3,000g and 4°C, and the plasma was immediately used.

**Separation and Assay of Angiotensins**

Angiotensins were extracted from 2 ml plasma by reversible adsorption to SepPak C18 cartridges (Waters) and were separated by reversed-phase high-performance liquid chromatography according to the method of Nussberger et al with some modifications as described previously. Concentrations of 125I-Ang I, 125I-Ang II, and their metabolites in collected chromatography fractions were measured directly in a 12-channel gamma counter (Multigamma 1260, LKB-Wallac, Turku, Finland). Fractions containing Ang I and Ang II were neutralized with sodium hydroxide and dried at reduced pressure. The Ang I and Ang II concentrations in these fractions were measured by radioimmunoassy. Characteristics of Ang I and Ang II antisera that were used in these radioimmunoassays and the way these assays were performed have been described previously. The lower limit of detection (2×SD difference from blank) was 1.0 fmol per tube for Ang I and 0.4 fmol per tube for Ang II. Recovery for Ang I and Ang II that were added to plasma was 85±7% and 84±8% (mean±SD, n=6), respectively. Similar values were obtained for 125I-Ang I and 125I-Ang II. Results of the angiotensin measurements were not corrected for incomplete recovery.

**Comparison Between Conversion of Angiotensin I to II and 125I-Angiotensin I to II In Vitro**

Solutions of 125I-Ang I (20×10^6 cpm/I), unlabeled Ang I (150 nM), the renin inhibitor CGP 29,287 (100 μM), and the ACE inhibitor captopril (25 mM) were prepared in water supplemented with bovine serum albumin (1 g/l). 125I-Ang I (50 μl) or unlabeled Ang I (50 μl) and CGP 29,287 (50 μl) were added to 7 ml fresh normal plasma. The mixture was incubated in the presence or absence of captopril (100 μl) at 37°C. Samples were taken from the incubate at 0, 1, 2, 5, 10, and 15 minutes to determine the half-life of added labeled and unlabeled Ang I. The half-life of added labeled and unlabeled Ang II was also measured.

In vitro conversion rates for 125I-Ang I and unlabeled Ang I were also compared by incubating 125I-Ang I (2×10^6 cpm/I) or unlabeled Ang I (10 pmol) with purified ACE (5 milliunits). Two ACE preparations were used, namely, ACE from rabbit lung (Sigma) or ACE from human kidney. Incubations were carried out at 37°C and pH 7.5 in the presence of 300 mM KCl, 1 mM ZnCl₂, 200 mM Tris, and 2 g/l bovine serum albumin. Total volume of incubate was 200 μl. Samples were centrifuged at 25 μl) were taken after 0, 1, 2, 5, 10, 20, and 30 minutes of incubation. The samples were diluted in 1 ml inhibitor solution containing 13 mM disodium EDTA and 75 μM of the ACE inhibitor lisinopril. Radiolabeled and unlabeled angiotensins were measured after extraction and separation.

**Subjects**

Regional metabolism of Ang I and Ang II was studied in 27 hypertensive subjects, 13 men and 14 women, aged 49.6±12.2 years (mean±SD). Combined infusions of 125I-Ang I and unlabeled Ang I were given to six subjects with EHT. Infusions of 125I-Ang I only were given to seven subjects with URAS and seven subjects with EHT. Infusions of 125I-Ang II were given to seven subjects with EHT. Subjects were not receiving antihypertensive treatment, except four subjects who received an infusion of 125I-Ang II while they were treated with the ACE inhibitor captopril, 25 mg twice daily.

All subjects were studied at the time they were undergoing renal vein renin sampling followed by renal angiography for diagnostic purposes. URAS was found in seven subjects. They proved to have a 60–90% stenosis of the renal artery. The remaining subjects showed no abnormalities on their angiogram and were diagnosed as having EHT.

**Infusion Protocol**

Angiotensin infusions were started after insertion of catheters into the abdominal aorta and the inferior caval vein via the femoral artery and vein with the Seldinger technique. Seven subjects with URAS and seven with EHT received an intravenous infusion of 125I-Ang I at a constant rate of 4.2±0.5×10^6 cpm/min (mean±SD; specific activity, approximately 74 TBq/mmol) for 20 minutes in the antecubital vein of the right arm. In the period between 8 and 20 minutes after the start of the infusion, when arterial and venous levels of 125I-Ang I and 125I-Ang II had reached a plateau, blood
samples were drawn from the veins of the left forearm, the leg, the liver, and both kidneys. Simultaneously with each venous sample, a sample from the aorta was taken. At 0.25, 0.5, 0.75, 1.10, and 1.45 minutes after discontinuation of the infusion, samples were taken from the aorta for determination of the biological half-life of infused angiotensins.

Six subjects with EHT received an intravenous infusion of a mixture of $^{125}$I-Ang I ($4.3 \pm 0.9 \times 10^6$ cpm/min) and unlabeled Ang I (0.8 \pm 0.1 nmol/min), and seven subjects with EHT received an intravenous infusion of $^{125}$I-Ang II (3.4 \pm 0.4 \times 10^6$ cpm/min; specific activity, approximately 74 TBq/nmol). Blood samples were taken simultaneously with each venous sample, a sample from the aorta was taken. At 0.25, 0.5, 0.75, 1.10, and 1.45 minutes after discontinuation of the infusion, samples were taken from the aorta for determination of the biological half-life of infused angiotensins.

Regional extraction of angiotensins I and II. The regional extraction ratio of $^{125}$I-Ang I, $^*E_1$, was defined as follows:

\[
E_1 = 1 - \frac{I_{\text{out}}}{I_{\text{in}}} \quad \text{(1)}
\]

in which $I_{\text{out}}$ and $I_{\text{in}}$ are the concentrations in inflowing (arterial) and outflowing (venous) plasma, respectively, at steady state during the $^{125}$I-Ang I infusion.

The regional extraction ratio of $^{125}$I-Ang II, $^*E_2$, was defined as follows:

\[
E_2 = 1 - \frac{I_{\text{out}}}{I_{\text{in}}} \quad \text{(2)}
\]

Regional degradation and conversion of angiotensin I. Regional extraction of arterially delivered $^{125}$I-Ang I at steady state during the $^{125}$I-Ang I infusion is the result of rapid metabolism, which occurs via two pathways, i.e., conversion into $^{125}$I-Ang II and degradation of $^{125}$I-Ang I into other peptides (Figure 1). Regional extraction of arterially delivered $^{125}$I-Ang II is the result of degradation only. Both conversion and degradation are enzymatic processes, and it may be assumed that the overall metabolism follows first-order reaction kinetics, according to the general formula $C_t = C_0 \cdot e^{-kt}$, in which $C_t$ is the concentration at time $t$ of the substance that is metabolized, $C_0$ is the concentration at time zero, and $k$ is the first-order reaction rate constant.

Regional metabolism of arterially delivered $^{125}$I-Ang I can be described by this formula as follows:

\[
^{125}$I-Ang I_{\text{out}} = \frac{-\ln(1 - ^*E_1)}{k_1} \quad \text{(3)}
\]

in which $^*k_1$ is the first-order rate constant for $^{125}$I-Ang I metabolism, and $t$ is the time the arterially delivered $^{125}$I-Ang I is exposed to the converting and degrading enzymes during the passage of blood. Thus, $^*k_1$ is given by the equation

\[
^*k_1 = -\ln(1 - ^*E_1) \quad \text{(4)}
\]

$^{125}$I-Ang I metabolism is the result of both degradation and conversion, and it may be assumed that

\[
^*k_2 = ^*k_1 + ^*k_3 \quad \text{(5)}
\]

Regional metabolism of arterially delivered $^{125}$I-Ang II at steady state during the $^{125}$I-Ang II infusion is the result of both degradation plus conversion, $^*k_3$, and assuming the first-order rate constant for $^{125}$I-Ang II metabolism $^{125}$I-Ang II degradation to be equal to the first-order rate constant for $^{125}$I-Ang I degradation, $^*k_2$ and $^*k_3$ can be expressed as a function of the $^{125}$I-Ang I and $^{125}$I-Ang II concentrations in inflowing and outflowing plasma:

\[
^*k_3 = -\ln(1 - ^*E_2) \quad \text{(6)}
\]

and, subsequently, $^*k_3$ can be calculated by subtracting $^*k_2$ from Equation 7, as given by Equation 8:

\[
^*k_3 = -\ln(1 - ^*E_2) = -\ln(1 - ^*E_1) - \ln(1 - ^*E_2) \quad \text{(7)}
\]

Similar to $^*k_1 + ^*k_3$ for $^{125}$I-Ang I metabolism (degradation plus conversion), $^*k_3$ for $^{125}$I-Ang II metabolism (degradation only) can be expressed as

\[
^*k_3 = -\ln(1 - ^*E_2) \quad \text{(8)}
\]

The $^*k_1$ values calculated with Equation 7 from results obtained during $^{125}$I-Ang I infusion were not different from the $^*k_3$ values calculated with Equation 9 from results obtained during $^{125}$I-Ang II infusion. These findings support the validity of calculating $^*k_3$ by using Equations 7 and 8, in which it is assumed that $^*k_3 = ^*k_1$.

We compared the metabolism of unlabeled Ang I with that of $^{125}$I-Ang I by combined infusion of the two peptides. As with $^*k_1$ and $^*k_3$, $^*k_1$ and $^*k_3$ for infused Ang I can be expressed as

\[
k_{1} = -\ln(1 - E_{1}) \quad \text{(9)}
\]

\[
k_{3} = -\ln(1 - E_{2}) \quad \text{(10)}
\]
and

\[ k_2t = \ln\left(\frac{\text{Ang I}_\text{out} + \text{Ang II}_\text{out}}{\text{Ang I}_\text{in} + \text{Ang II}_\text{in}}\right) \]

\[ -\ln(\text{Ang I}_\text{out}/\text{Ang I}_\text{in}) \quad (11) \]

in which \( \text{Ang I}_\text{in} \) and \( \text{Ang I}_\text{out} \) are the concentrations of infused Ang I in inflowing (arterial) and outflowing (venous) plasma, and \( \text{Ang II}_\text{in} \) and \( \text{Ang II}_\text{out} \) are the concentrations of Ang II derived from conversion of infused Ang I.

The concentration of infused Ang I and the concentration of Ang II derived from infused Ang I can be calculated by subtracting the levels of endogenous Ang I and II measured before infusion from the levels measured during infusion. Ang I infusion causes extra Ang II formation, which reduces renin secretion from the kidney and consequently reduces the level of endogenous Ang I. However, during the short infusion period, the levels of endogenous Ang I, measured at 2 minutes after the discontinuation of the combined infusion, were only marginally suppressed (see "Results"). We therefore did not correct for this small reduction in endogenous Ang I.

Analogous to \( *k_{3t} \), \( k_{3t} \) for infused Ang II can be expressed as

\[ k_{3t} = -\ln(\text{Ang II}_\text{out}/\text{Ang II}_\text{in}) \quad (12) \]

From the results obtained during the combined \(^{125}\)I-Ang I and Ang I infusion, it appeared that the values of \( k_{3t} \) for infused Ang I, which were calculated with Equation 10, were not significantly different from the values of \( *k_{3t} \) for \(^{125}\)I-Ang I, which were calculated with Equation 7 (see "Results"). However, \( k_{3t} \) for infused Ang I was 0.65 times \( *k_{3t} \) for \(^{125}\)I-Ang I. Thus,

\[ k_{3t} = *k_{3t} \quad (13) \]

and

\[ k_{3t} = 0.65 \times *k_{3t} \quad (14) \]

The fractional regional destruction of arterially delivered endogenous Ang I is defined as

\[ \text{Fractional Ang I degradation} = E_1 \times k_{1t}/(k_{1t}+k_{3t}) \quad (15) \]

The fractional regional conversion of arterially delivered endogenous Ang I is defined as

\[ \text{Fractional Ang I conversion} = E_1 \times k_{2t}/(k_{1t}+k_{3t}) \quad (16) \]

Fractional Ang I degradation and conversion could be calculated from the results obtained during infusion of \(^{125}\)I-Ang I alone as follows: First, \( *k_{1t} \) and \( *k_{3t} \) were calculated according to Equations 7 and 8; then, Equations 13 and 14 were used to calculate \( k_{1t} \) and \( k_{3t} \); and finally, these calculated values of \( k_{1t} \) and \( k_{3t} \) were used to obtain \( E_1 \) with the equation

\[ k_{1t} + k_{3t} = -\ln(1-E_1) \quad (17) \]

which is analogous to Equation 6.

Sources of endogenous angiotensins I and II in regional venous plasma. Endogenous Ang I in regional venous plasma originates from arterial delivery and de novo production. De novo production of Ang I occurs at tissue sites where part of this locally produced Ang I reaches the circulation.\(^{14,15}\) In addition, some de novo production of Ang I occurs in circulating plasma by PRA, during the passage of blood from the arterial to the venous side of the vascular bed.

Regional venous Ang I derived from arterial delivery could be calculated from the results obtained during infusion of \(^{125}\)I-Ang I alone as follows:

\[ \text{Ang I}_\text{out} = \frac{\text{Ang I}_\text{in} \times (1-E_1)}{1+*k_{3t}} \quad (18) \]

in which \( \text{Ang I}_\text{in} \) is the concentration of endogenous Ang I in inflowing (arterial) plasma, and \( E_1 \) is given by Equation 17.

Regional venous Ang II derived from regional production by circulating PRA was calculated as

\[ \text{Ang I}_\text{out} = \text{PRA} \times \text{blood transit time} \quad (19) \]

This equation does not take into account the regional metabolism of Ang I and therefore leads to an overestimation of the level of regional venous Ang I that is regionally produced by circulating PRA.

Endogenous Ang II in regional venous plasma also originates from arterial delivery and de novo production. De novo production depends, at least in part, on the conversion of arterially delivered Ang I. Regional venous Ang II derived from arterial delivery was calculated as

\[ \text{Ang II}_\text{out} = \frac{\text{Ang II}_\text{in} \times (1-E_1)}{1+*k_{3t}} \quad (20) \]

in which \( \text{Ang II}_\text{in} \) is the concentration of endogenous Ang II in inflowing (arterial) plasma, and \( E_2 \) is given by the equation

\[ k_{3t} = -\ln(1-E_2) \quad (21) \]

which is analogous to Equation 6. With Equation 21, \( E_2 \) could be calculated from the results obtained during infusion of \(^{125}\)I-Ang I alone by assuming that \( k_{3t} = *k_{3t} \) (see "Results").

According to the reaction scheme shown in Figure 1 and assuming \( k_{3t} = *k_{1t} \) (see "Results"), regional venous Ang II derived from conversion of arterially delivered Ang I was calculated as

\[ \text{Ang II}_\text{out} = \frac{\text{Ang II}_\text{in}}{1+*k_{3t}} \quad (22) \]

Statistical Evaluation

Statistical differences were evaluated with the paired or unpaired Student's \( t \) test. Whenever data were not normally distributed, the nonparametric Wilcoxon signed rank test was used instead. Significance was assumed at a value of \( p<0.05 \).

Results

In Vitro Studies

Conversion of \(^{125}\)I-angiotensin I to II versus angiotensin 1 to II in plasma. Freshly obtained normal human plasma, to which the renin inhibitor CGP 29,287 had been added, was used in these experiments (see "Methods"). The concentration of the inhibitor was high enough to block Ang I generation completely. Experiments were carried out in triplicate. In the absence of ACE inhibition, that is, without the addition of captopril (see "Methods"), the half-life of \(^{125}\)I-Ang I in plasma ranged from 1.7 to 2.6 minutes, and
the half-life of Ang I that had been added to the plasma ranged from 2.8 to 3.2 minutes. The first-order elimination rate constant for $^{125}$I-Ang I, $k_1$, was 0.35 min$^{-1}$ (mean, $n=3$), and the first-order elimination rate constant for Ang I, $k_2$, was 0.23 min$^{-1}$.

In the presence of the ACE inhibitor captopril, the plasma half-life ranged from 11 to 16 minutes for $^{125}$I-Ang I and from 13 to 18 minutes for added Ang I. In the presence of captopril, conversion is virtually zero, so that under these conditions the first-order elimination rate constants, $k_1$ and $k_2$, are equal to the first-order degradation rate constants, $k_1$ and $k_2$, respectively. The first-order degradation rate constant for $^{125}$I-Ang I, $k_1$, was 0.046 min$^{-1}$, and the first-order degradation rate constant for Ang I, $k_2$, was 0.051 min$^{-1}$. The $k_1$-to-$k_2$ ratio was 1.1.

The half-life in plasma of $^{125}$I-Ang II and added Ang II ranged from 5 to 10 minutes. This value is very similar to the half-life in plasma, in the presence of captopril, of $^{125}$I-Ang I and Ang I. This indicates that the degradation rate constants, $k_1$ for Ang I, $k_2$ for $^{125}$I-Ang II, $k_3$ for Ang II, and $k_4$ for $^{125}$I-Ang II, all have approximately the same value.

Conversion of $^{125}$I-angiotensin I to II versus angiotensin I to II by purified tissue angiotensin converting enzyme. Purified ACE isolated from human kidney and a commercial preparation of ACE from rabbit lung were used in these experiments. The first-order rate constant, $k_2$, for Ang I to-II conversion was compared with the first-order rate constant for $^{125}$I-Ang I, $k_1$, and Ang I to-II conversion, $k_2$, was 0.18 min$^{-1}$. The $k_2$-to-$k_2$ ratio was 0.6.

The half-life in plasma of $^{125}$I-Ang I and added Ang I ranged from 11 to 15 minutes. This value is very similar to the half-life in plasma, in the presence of captopril, of $^{125}$I-Ang I and Ang I. This indicates that the degradation rate constants, $k_1$ for Ang I, $k_2$ for $^{125}$I-Ang II, $k_3$ for Ang II, and $k_4$ for $^{125}$I-Ang II, all have approximately the same value.

Conversion of $^{125}$I-angiotensin I to II versus angiotensin I to II by purified tissue angiotensin converting enzyme. Purified ACE isolated from human kidney and a commercial preparation of ACE from rabbit lung were used in these experiments. The first-order rate constant, $k_2$, for Ang I to-II conversion was compared with the first-order rate constant for $^{125}$I-Ang I, $k_1$, and Ang I to-II conversion, $k_2$, was 0.18 min$^{-1}$. The $k_2$-to-$k_2$ ratio was 0.6.

The half-life in plasma of $^{125}$I-Ang II and added Ang II ranged from 5 to 10 minutes. This value is very similar to the half-life in plasma, in the presence of captopril, of $^{125}$I-Ang I and Ang I. This indicates that the degradation rate constants, $k_1$ for Ang I, $k_2$ for $^{125}$I-Ang II, $k_3$ for Ang II, and $k_4$ for $^{125}$I-Ang II, all have approximately the same value.

Conversion of $^{125}$I-angiotensin I to II versus angiotensin I to II by purified tissue angiotensin converting enzyme. Purified ACE isolated from human kidney and a commercial preparation of ACE from rabbit lung were used in these experiments. The first-order rate constant, $k_2$, for Ang I to-II conversion was compared with the first-order rate constant for $^{125}$I-Ang I, $k_1$, and Ang I to-II conversion, $k_2$, was 0.18 min$^{-1}$. The $k_2$-to-$k_2$ ratio was 0.6.

The half-life in plasma of $^{125}$I-Ang I and added Ang I ranged from 11 to 15 minutes. This value is very similar to the half-life in plasma, in the presence of captopril, of $^{125}$I-Ang I and Ang I. This indicates that the degradation rate constants, $k_1$ for Ang I, $k_2$ for $^{125}$I-Ang II, $k_3$ for Ang II, and $k_4$ for $^{125}$I-Ang II, all have approximately the same value.

Conversion of $^{125}$I-angiotensin I to II versus angiotensin I to II by purified tissue angiotensin converting enzyme. Purified ACE isolated from human kidney and a commercial preparation of ACE from rabbit lung were used in these experiments. The first-order rate constant, $k_2$, for Ang I to-II conversion was compared with the first-order rate constant for $^{125}$I-Ang I, $k_1$, and Ang I to-II conversion, $k_2$, was 0.18 min$^{-1}$. The $k_2$-to-$k_2$ ratio was 0.6.

The half-life in plasma of $^{125}$I-Ang I and added Ang I ranged from 11 to 15 minutes. This value is very similar to the half-life in plasma, in the presence of captopril, of $^{125}$I-Ang I and Ang I. This indicates that the degradation rate constants, $k_1$ for Ang I, $k_2$ for $^{125}$I-Ang II, $k_3$ for Ang II, and $k_4$ for $^{125}$I-Ang II, all have approximately the same value.

Conversion of $^{125}$I-angiotensin I to II versus angiotensin I to II by purified tissue angiotensin converting enzyme. Purified ACE isolated from human kidney and a commercial preparation of ACE from rabbit lung were used in these experiments. The first-order rate constant, $k_2$, for Ang I to-II conversion was compared with the first-order rate constant for $^{125}$I-Ang I, $k_1$, and Ang I to-II conversion, $k_2$, was 0.18 min$^{-1}$. The $k_2$-to-$k_2$ ratio was 0.6.

The half-life in plasma of $^{125}$I-Ang I and added Ang I ranged from 11 to 15 minutes. This value is very similar to the half-life in plasma, in the presence of captopril, of $^{125}$I-Ang I and Ang I. This indicates that the degradation rate constants, $k_1$ for Ang I, $k_2$ for $^{125}$I-Ang II, $k_3$ for Ang II, and $k_4$ for $^{125}$I-Ang II, all have approximately the same value.
values calculated from the data obtained during the infusion of $^{125}$I-Ang II alone.

**Regional Production of Angiotensins I and II**

Arterial and venous plasma levels of endogenous angiotensins I and II. Tables 3 and 4 give the arterial and venous plasma levels of endogenous Ang I and II in the subjects who received an infusion of $^{125}$I-Ang I (not combined with Ang I). The venous level of Ang I in the forearm was not significantly different from the arterial level, both in the subjects with URAS and in EHT. Venous levels of Ang I in the leg were significantly higher than arterial Ang I in URAS but not in EHT. There was no arteriovenous gradient of Ang II across the forearm and leg in both URAS and EHT.

In the subjects with URAS, renal venous Ang I on the affected side was approximately three times higher than arterial Ang I, whereas on the unaffected side, renal venous Ang I was similar to arterial Ang I. Thus, there was net release of Ang I from the affected kidney into the systemic circulation. On both the affected and unaffected sides in these subjects, renal venous Ang II was lower than arterial Ang II, indicating net extraction of Ang II from the systemic circulation. In EHT, renal venous Ang I on both sides was somewhat higher than arterial Ang I, whereas renal venous Ang II was lower than arterial Ang II.

In both groups of subjects, the levels of Ang I and Ang II in the hepatic vein were much lower than in the artery, illustrating the high degree of extraction of

---

**Table 1. Regional Vein-to-Artery Ratios in Subjects With Essential Hypertension**

<table>
<thead>
<tr>
<th>Region</th>
<th>Single infusion, $^{125}$I-Ang I (n=7)</th>
<th>Combined infusion, $^{125}$I-Ang I and Ang I (n=6)</th>
<th>Single infusion, $^{125}$I-Ang II (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1−V/A $^{125}$I-Ang I</td>
<td>1−V/A $^{125}$I-Ang I and Ang I</td>
<td>1−V/A $^{125}$I-Ang II</td>
</tr>
<tr>
<td>Forearm</td>
<td>0.64±0.18</td>
<td>0.67±0.19</td>
<td>0.65±0.20</td>
</tr>
<tr>
<td>Leg</td>
<td>0.84±0.06</td>
<td>0.86±0.06</td>
<td>0.79±0.13</td>
</tr>
<tr>
<td>Right kidney</td>
<td>0.88±0.05</td>
<td>0.85±0.04</td>
<td>0.84±0.10</td>
</tr>
<tr>
<td>Left kidney</td>
<td>0.85±0.05</td>
<td>0.87±0.03</td>
<td>0.82±0.16</td>
</tr>
<tr>
<td>Liver and gut</td>
<td>0.98±0.02</td>
<td>0.97±0.02</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Ang I, angiotensin I; Ang II, angiotensin II; V/A, vein-to-artery ratio; n.d., not determined. Values are mean±SD.
systemically delivered Ang I and Ang II across the hepatomesenteric vascular bed.

Regional venous plasma levels of de novo–produced angiotensin I. The values of \( k_1 \) and \( k_2 \) in the subjects who received an infusion of \(^{125}\)I-Ang I (not combined with Ang I) were calculated from the data presented in Tables 3 and 4 by using Equations 7 and 8. These \( k_1 \) and \( k_2 \) values were then used for calculating the venous level of Ang I derived from arterial delivery, according to Equation 18. Subtraction of this fraction from the level that was actually measured gives the venous level of de novo–produced Ang I. Results are shown in Figure 3. Most of de novo–produced venous Ang I in forearm and leg could not be accounted for by circulating PRA, both in the group with URAS and in EHT. In the subjects with URAS, the venous plasma level of intrarenally de novo–produced Ang I was higher on the affected side than on the unaffected side and could also not be accounted for by circulating PRA. In contrast, in the hepatic vein, most of Ang I that is de novo–produced in the hepatomesenteric vascular bed could be accounted for by circulating PRA.

Regional venous plasma levels of de novo–produced angiotensin II. The values of \( k_1 \) and \( k_2 \) in the subjects who received an infusion of \(^{125}\)I-Ang I (not combined with Ang I) were also used for calculating the fractional regional conversion and degradation of arterially delivered endogenous Ang I (Equations 15 and 16) and for calculating the venous level of Ang II derived from arterial delivery (Equation 20). Subtraction of this level from the level that was actually measured (see Tables 3 and 4) gives the venous level of de novo–produced Ang II. Results are shown in Figures 4 and 5. In the forearm, 20%, and in the leg, 30% of arterially delivered Ang I was converted to Ang II, confirming that a substantial part of the conversion of circulating Ang I occurs outside the lungs. There was no difference in fractional conversion of arterially delivered Ang I in the forearm and leg between the subjects with URAS and EHT. We could not demonstrate Ang I-to-II conversion in the renal and hepatomesenteric vascular beds in both groups.

Release of locally produced Ang II into the venous plasma in the forearm and leg was 25–30% of the release of locally produced Ang I, and by far most of the de novo production of Ang II could be accounted for by conversion of arterially delivered Ang I. The venous level of de novo–produced Ang II that could not be accounted for by this source was less than 1 pmol/l in the forearm and leg. Our estimate of the venous level of

<table>
<thead>
<tr>
<th>TABLE 2. Regional Degradation and Conversion Constants in Subjects With Essential Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Forearm</td>
</tr>
<tr>
<td>Leg</td>
</tr>
<tr>
<td>Right kidney</td>
</tr>
<tr>
<td>Left kidney</td>
</tr>
<tr>
<td>Liver and gut</td>
</tr>
</tbody>
</table>

Ang I, angiotensin I; Ang II, angiotensin II; \( k_1 \) and \( k_2 \), first-order rate constant for \(^{125}\)I-Ang I and Ang I degradation; \( k_1 \) and \( k_2 \), first-order rate constant for \(^{125}\)I-Ang I-to-II and Ang I-to-II conversion; \( k_2 \), first-order rate constant for \(^{125}\)I-Ang II degradation; n.d., not determined. Values are mean±SD.

*\( p < 0.05 \) for difference between \( k_1 \) and \( k_2 \) during combined infusion.

**\( p < 0.05 \) for difference between Ang I and Ang II plasma levels.

***\( p < 0.05 \) for difference between \( k_1 \) during combined infusion.

\( p < 0.05 \) for difference between Ang I and Ang II plasma levels (Wilcoxon signed rank test).

<table>
<thead>
<tr>
<th>TABLE 3. Plasma Levels of Plasma Renin Activity, Angiotensins I and II, and (^{125})I-Angiotensins I and II Across Various Vascular Beds During (^{125})I-Angiotensin I Infusion in Seven Subjects With Essential Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular bed</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>Aorta</td>
</tr>
<tr>
<td>Antecubital vein</td>
</tr>
<tr>
<td>Aorta</td>
</tr>
<tr>
<td>Femoral vein</td>
</tr>
<tr>
<td>Aorta</td>
</tr>
<tr>
<td>Right renal vein</td>
</tr>
<tr>
<td>Aorta</td>
</tr>
<tr>
<td>Left renal vein</td>
</tr>
<tr>
<td>Aorta</td>
</tr>
<tr>
<td>Hepatic vein</td>
</tr>
</tbody>
</table>

PRA, plasma renin activity; Ang I, angiotensin I; Ang II, angiotensin II. Data are median values and ranges.

*\( p < 0.05 \) for difference between aortic and venous plasma levels (Wilcoxon signed rank test).
De novo-produced Ang II includes Ang II formed during the time taken to collect blood from the antecubital and femoral veins and also Ang II produced by conversion of Ang I that is generated by circulating PRA during passage of blood from artery to vein. However, the contribution of these two sources to our estimates of the venous level of de novo-produced Ang II is small. As shown in Figure 3, the contribution of circulating PRA to de novo-produced venous Ang I in the forearm and leg accounts for less than 15-25% of total de novo-produced venous Ang I. In the forearm, 20%, and in the leg, 30% of arterially delivered Ang I was converted to Ang II. It is unlikely that Ang I generated by circulating PRA is subject to the same degree of regional conversion as arterially delivered Ang I, because the former is exposed to ACE for a shorter time than the latter. Thus, the contribution of circulating PRA to de novo-produced venous Ang II in the limbs could be calculated to be less than 6%.

In the kidney, the release of locally produced Ang II was 10-15% of the release of locally produced Ang I, and in contrast to the limbs, virtually none of de novo-produced venous Ang II in the kidney could be accounted for by conversion of arterially delivered Ang I. However, the renal venous level of de novo-produced Ang II was less than 2 pmol/l in EHT and less than 4 pmol/l on the affected side and less than 1 pmol/l on the unaffected side in URAS. Again, these figures may be overestimates because some Ang II was formed during the time it took to collect blood from the renal veins (approximately 15 seconds). At the time the blood is collected, mixing with the inhibitor solution in the syringe is not complete. Given a first-order rate constant for Ang I-to-II conversion in plasma of 0.18 min⁻¹ and a rate constant of only 0.05 min⁻¹ for Ang I and Ang II degradation (see results of the in vitro experiments), and given the high renal venous levels of Ang I, particularly on the affected side in URAS, the measured Ang II levels in renal vein samples may exceed the "true" Ang II levels by 1-3 pmol/l.

The levels of de novo-produced Ang I in the hepatic vein were low, probably because of the highly effective extraction of Ang I by the liver. Nearly complete extraction of Ang II may also explain the low Ang II levels in the hepatic vein. These levels were too low to make a reliable estimate of the fraction derived from de novo production in the hepatomesenteric vascular bed. Thus, it appears that, in the limbs and the kidney, little of Ang I produced at tissue sites reaches the blood in the regional veins in the form of Ang II and that most Ang II present in the circulation originates from blood-borne Ang I.

**Discussion**

Little is known in humans about the contribution of extrapulmonary Ang I-to-II conversion to the plasma level of Ang II. It has been reported that the plasma Ang II-to-I concentration ratio is not reduced in human subjects during extrapulmonary bypass with extracorporeal circulation, which may suggest that extrapulmonary conversion of arterially delivered Ang I is the most important source of circulating Ang II. 19 It should be noted, however, that the Ang II-to-I concentration ratio in plasma is not only dependent on the degree of conversion of blood-borne Ang I; it also depends on Ang I and II degradation and Ang I production, as well as on Ang II production from sources other than conversion of blood-borne Ang I.

The present study enabled us to measure directly the degree of conversion of blood-borne Ang I in extrapulmonary vascular beds. Our estimates of regional Ang I-to-II conversion were based on data obtained during systemic infusion of 125I-Ang I. We accounted for the somewhat higher in vivo conversion rates of radiolabeled Ang I as opposed to unlabeled Ang I. Our in vitro experiments indicate that this difference in conversion rate is probably due to the difference in the Vmax/Km ratio of ACE for the two substrates. This explains why the arterial and venous 125I-Ang II/125I-Ang I concentration ratios during 125I-Ang I infusion were higher than the corresponding endogenous Ang II/Ang I ratios (see Tables 3 and 4). This, however, cannot be the full explanation because the discrepancy is too big. Indeed, a discrepancy was still observed when unlabeled Ang I was infused. From the data shown in Figure 2 it can be derived that, for each subject receiving an infusion of unlabeled Ang I, the Ang II/Ang I ratio in the aorta was higher during the infusion than before. This difference clearly illustrates that a higher proportion of

---

**Table 4. Plasma Levels of Plasma Renin Activity, Angiotensins I and II, and 125I-Angiotensins I and II Across Various Vascular Beds During 125I-Angiotensin I Infusion in Seven Subjects With Unilateral Renal Artery Stenosis**

<table>
<thead>
<tr>
<th>Vascular bed</th>
<th>Plasma level</th>
<th>PRA (pmol . l⁻¹ . min⁻¹)</th>
<th>Ang I (pmol/l)</th>
<th>Ang II (pmol/l)</th>
<th>125I-Ang I (cpm/l x 10⁶)</th>
<th>125I-Ang II (cpm/l x 10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>7</td>
<td>21 (7.2-64)</td>
<td>16 (5.6-49)</td>
<td>8.9 (3.0-22)</td>
<td>306 (120-621)</td>
<td>1,003 (712-1,777)</td>
</tr>
<tr>
<td>Antecubital vein</td>
<td>7</td>
<td>20 (7.7-72)</td>
<td>16 (5.4-56)</td>
<td>8.7 (2.7-21)</td>
<td>331 (127-709)</td>
<td>996 (670-1,906)</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>7</td>
<td>21 (7.5-77)</td>
<td>26 (6.3-63)*</td>
<td>8.9 (2.9-16)</td>
<td>39 (16-85)*</td>
<td>460 (290-661)*</td>
</tr>
<tr>
<td>Renal vein (affected kidney)</td>
<td>7</td>
<td>21 (7.5-77)</td>
<td>21 (5.4-45)</td>
<td>11 (4-12)</td>
<td>310 (134-709)</td>
<td>998 (735-1,906)</td>
</tr>
<tr>
<td>Aorta</td>
<td>7</td>
<td>21 (7.5-77)</td>
<td>50 (10-119)*</td>
<td>68 (8.5-113)*</td>
<td>6.1 (1.9-11)*</td>
<td>56 (20-106)*</td>
</tr>
<tr>
<td>Renal vein (unaffected kidney)</td>
<td>7</td>
<td>28 (7.5-71)</td>
<td>15 (5.4-58)</td>
<td>3.4 (0.9-8.5)*</td>
<td>77 (22-124)*</td>
<td>234 (106-323)*</td>
</tr>
<tr>
<td>Aorta</td>
<td>4</td>
<td>14 (7.0-39)</td>
<td>9.9 (5.5-23)</td>
<td>7.1 (4.5-8.7)</td>
<td>217 (196-384)</td>
<td>1,056 (705-1,203)</td>
</tr>
</tbody>
</table>

PRA, plasma renin activity; Ang I, angiotensin I; Ang II, angiotensin II. Data are median values and ranges.

*p<0.05 for difference between aortic and venous plasma levels (Wilcoxon signed rank test).
Exogenous Ang I is passed along the sites where the conversion is occurring. Circulating Ang I is probably converted mainly at the level of the arterioles, whereas Ang I is formed at all levels of the circulation. In addition, Ang I formed in the tissues may reach the circulation mainly at the level of the capillaries and venules (see also last paragraph of "Discussion").

Forearm and leg converted 20-30% of arterially delivered Ang I. We found little or no conversion of arterially delivered Ang I in the kidney and in the hepatomesenteric vascular bed. 125I-Ang I infusion experiments in pigs have shown that the venous plasma levels of 125I-Ang I and II in the leg during these infusions are similar to those in mixed venous plasma. The cardiac output of plasma in our subjects is assumed to be 3 l/min and if it is assumed that during the systemic infusion of 125I-Ang I the venous plasma levels of 125I-Ang I and II we measured in the leg are representative of the levels in mixed venous plasma, it can be calculated from our results (with Equation 16) that 40-50% of Ang I delivered to the central circulation is converted to Ang II during a single passage of blood from the right atrium to the aorta, whereas 20-30% of Ang I delivered to the systemic circulation is converted to Ang II during a single passage of blood from the aorta to the right atrium. Thus, because the plasma levels of endogenous Ang I in the aorta have been reported to be only 20% lower than in the right atrium and the peripheral veins,21 our results indicate that 30-40% of the conversion of blood-borne Ang I occurs outside the lungs. In a similar way (by using Equation 15), it can be calculated that only about 15% of Ang I delivered to the central circulation is degraded into peptides other than Ang II during a single passage of blood from the right atrium to the aorta, whereas 35-50% of Ang I delivered to the systemic circulation is degraded during a single passage of blood from the aorta to the right atrium, indicating that at least 70-80% of the degradation of blood-borne Ang I occurs outside the lungs. Our estimates of Ang I conversion and degradation in the central vascular bed are in good agreement with results in numerous studies showing in the lungs approximately 50% conversion of arterially delivered Ang I and little degradation of arterially delivered Ang I and II,11,12,21-29

We were unable to detect any Ang I-to-II conversion in the kidneys both in URAS and in EHT. In URAS on the affected side and in EHT on both sides, our
ESSENTIAL HYPERTENSION

Fractional metabolism

1.0
0.8
0.6
0.4
0.2
0.0
Forearm  Leg  Right  Kidney  Left  Kidney  Liver and Gut

FIGURE 4. Bar graphs show fractional conversion and degradation (mean values, SD) of arterially delivered angiotensin I in different vascular beds in subjects with essential hypertension and unilateral renal artery stenosis.

UNILATERAL RENAL ARTERY STENOSIS

Fractional metabolism

1.0
0.8
0.6
0.4
0.2
0.0
Forearm  Leg  Affected  Kidney  Unaffected  Kidney  Liver and Gut

estimates of conversion yielded even negative values. A somewhat higher degradation rate for Ang II than for Ang I in the kidney could explain these negative values of conversion. In our calculations of regional Ang I-to-II conversion and Ang I degradation, the first-order rate constant of Ang I degradation, \( k_1 \), was assumed to be equal to the first-order rate constant of Ang II degradation, \( k_3 \). Although the results of our infusion studies indicated that \( k_1 \) and \( k_3 \) are likely to be similar, some differences may exist. In dogs, 20% of \(^{125}\text{I}-\text{Ang I} \) given as a bolus injection into the renal artery was converted to \(^{125}\text{I}-\text{Ang II} \) during a single passage through the kidney, and reduction of renal blood flow by renal artery constriction did not change the fractional conversion of arterially delivered \(^{125}\text{I}-\text{Ang I} \). Given the possibility that, as we found in humans, \(^{125}\text{I}-\text{Ang I} \) is more rapidly converted than unlabeled Ang I, the intrarenal fractional conversion of arterially delivered Ang I was probably somewhat overestimated in these dogs. In addition, the figure of 20% conversion in the dog kidney was based on the amount of \(^{125}\text{I}-\text{Ang II} \) that was recovered in renal venous blood collected during a 1-minute period after intra-arterial injection of \(^{125}\text{I}-\text{Ang I} \). Because conversion in blood in vitro is not negligible, some \(^{125}\text{I}-\text{Ang II} \) in samples from the renal vein may have been generated during the collection of blood. In the isolated perfused rat kidney, Ang I had to be infused in a 50 times higher molar dose than Ang II to induce the same vasoconstrictor response. Thus, there was apparently some intrarenal conversion of arterially delivered Ang I, but only to a very limited degree.

The low degree of conversion of arterially delivered Ang I in the kidney is unexpected in view of the high concentration of Ang II found in renal tissue and the profound renal effects of ACE inhibition. It is possible that the kidney is highly responsive even to the small quantities of Ang II that are intrarenally formed by conversion of arterially delivered Ang I. However, it seems more likely that Ang I-to-II conversion in the kidney occurs in a compartment that is accessible to ACE inhibitors but does not readily equilibrate with Ang I and II from the circulation.

From previous \(^{125}\text{I}-\text{Ang I} \) infusion studies in hypertensive subjects who were all receiving ACE inhibitor treatment, we concluded that a major part of venous plasma Ang I in forearm, leg, and kidney is produced de
novo in these vascular beds, that part of this de novo production cannot be accounted for by circulating PRA, and that in the kidney in situ synthesized renin is an important determinant of local Ang I production (i.e., Ang I production not in circulating plasma). The present study, conducted in hypertensive subjects who were not receiving ACE inhibitor treatment, confirms these conclusions. In addition, the present study shows also that a major part of venous plasma Ang II in forearm and leg originates from regional de novo production and not from arterial delivery. In the limbs, most if not all de novo–produced venous Ang II was derived from arterially delivered Ang I. Also, in the lungs, the venous level of de novo–produced Ang II could be fully accounted for by conversion of arterially delivered Ang I.

In the kidney, the situation is clearly different. Whereas in forearm and leg the level of Ang II in the veins was similar to that in the artery, venous Ang II in the kidney was only half that of arterial Ang II. Moreover, whereas in forearm and leg virtually all venous Ang II could be accounted for by arterial Ang II delivery and regional conversion of arterially delivered Ang I, part of venous Ang II in the kidney appeared to come from a different source. Intrarenal Ang I production by circulating PRA followed by intrarenal conversion to Ang II could not account for this source because of the short blood transit time and the low conversion rate of blood-borne Ang I in the kidney. The calculated levels of intrarenally produced Ang II in the renal vein were, however, low, and as discussed in “Results,” most of it may be due to Ang I-to-II conversion in the samples during their collection. Thus, little intrarenally produced Ang II is released into the circulation via the renal vein. Also, little Ang II that might be produced in the hepatomesenteric vascular bed appears to be released into the circulation via the hepatic vein.

Our data, taken together, provide little evidence for the production of circulating Ang II via pathways other than conversion of blood-borne Ang I. In the vascular beds we studied, little of the Ang I that is formed in situ at tissue sites reaches the venous blood of these beds in the form of Ang II. In the renal and hepatomesenteric vascular beds, this might be due to a lack of vascular endothelial ACE activity, as indicated by the low degree of conversion of Ang I that is arterially delivered to these vascular beds. On the other hand, in the limbs there is marked conversion of arterially delivered Ang I, and the locally formed Ang I appears to enter the blood at a level distal to the site where arterially delivered Ang I.
I is converted to Ang II, so that this conversion site is bypassed. Ang I formed at tissue sites probably enters the circulation at the level of the capillaries or venules, whereas Ang I-to-II conversion occurs at the level of the arterioles. This explanation would fit with the hypothesis that the vascular endothelium is the physiologically relevant production site of circulating Ang II.\(^{35}\) Our experiments cannot exclude the possibility that part of the in situ synthesized Ang I is converted before it enters the circulation and that the Ang II thus formed remains in the tissues and does not easily reach the circulating blood. At any rate, the results of the present study illustrate the high degree of compartmentalization of Ang I and II production.

Acknowledgments

We thank Angelique van der Houwen, Rene de Bruin, Carolien van Gelderen, and Nelleke Bos-Sonneveld for their skillful technical assistance, and Carla Swaab for the preparation of the figures.

References

Regional angiotensin II production in essential hypertension and renal artery stenosis.
P J Admiraal, A H Danser, M S Jong, H Pieterman, F H Derkx and M A Schalekamp

Hypertension. 1993;21:173-184
doi: 10.1161/01.HYP.21.2.173

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/21/2/173

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

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

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