Human Vascular Renin-Angiotensin System and Its Functional Changes in Relation to Different Sodium Intakes

Maria Boddi, Loredana Poggesi, Mirella Coppo, Nicoletta Zarone, Simona Sacchi, Chechi Tania, Gian Gastone Neri Serneri

Abstract—A growing body of evidence supports the existence of a tissue-based renin-angiotensin system (RAS) in the vasculature, but the functional capacity of vascular RAS was not investigated in humans. In 28 normotensive healthy control subjects, the metabolism of angiotensins through vascular tissue was investigated in normal, low, and high sodium diets by the measurement of arterial-venous gradient of endogenous angiotensin (Ang) I and Ang II in two different vascular beds (forearm and leg), combined with the study of $^{125}$I-Ang I and $^{125}$I-Ang II kinetics. In normal sodium diet subjects, forearm vascular tissue extracted 36±6% of $^{125}$I-Ang I and 30±5% of $^{125}$I-Ang II and added 14.9±5.1 fmol·100 mL$^{-1}$·min$^{-1}$ of de novo formed Ang I and 6.2±2.8 fmol·100 mL$^{-1}$·min$^{-1}$ of Ang II to antecubital venous blood. Fractional conversion of $^{125}$I-Ang I through forearm vascular tissue was about 12%. Low sodium diet increased (P<.01) plasma renin activity, whereas de novo Ang I and Ang II formation by forearm vascular tissue became undetectable. Angiotensin degradation (33±7% for Ang I and 30±7% for Ang II) was unchanged, and vascular fractional conversion of $^{125}$I-Ang I decreased from 12% to 6% (P<.01). In high sodium diet subjects, plasma renin activity decreased, and de novo Ang I and Ang II formation by forearm vascular tissue increased to 22 and 14 fmol·100 mL$^{-1}$·min$^{-1}$, respectively (P<.01). Angiotensin degradation did not significantly change, whereas fractional conversion of $^{125}$I-Ang I increased from 12% to 20% (P<.01). Leg vascular tissue functional activities of RAS paralleled those of forearm vascular tissue both at baseline and during different sodium intake. These results provide consistent evidence for the existence of a functional tissue-based RAS in vascular tissue of humans. The opposite changes of plasma renin activity and vascular angiotensin formation indicate that vascular RAS is independent from but related to circulating RAS. (Hypertension. 1998;31:836–842.)

Key Words: angiotensin • renin-angiotensin system • vessels

In the last few years, the classic concept of the RAS as a solely circulating system has undergone fundamental modifications due to the recent molecular and biochemical approaches to the physiology of the RAS that have provided evidence for the existence of intrinsic RAS in multiple tissues and organs.1–3 Local production of Ang I and Ang II has been demonstrated in cell culture and in organ preparations,4–6 and local RAS have been proposed in brain, heart, and kidney.

Ang II is provided with multiple vascular actions including direct vasoconstrictor effect, enhancement of sympathetic adrenergic transmission,7,8 and myogenic and trophic actions in the vasculature.9–11 Therefore, a vascular RAS is of special interest because its existence may have important implications in the pathophysiology of diseases such as hypertension, atherosclerosis, restenosis after angioplasty, and heart failure.

Indirect evidence for a local RAS has been derived from whole animals’ isolated organs4,12–14 and from cultured arterial smooth muscle cells from dog and rat, which have been found to contain renin,15 angiotensinogen,16 angiotensin receptors,17 and ACE.18 ACE activity has been immunohistochemically detected in smooth muscle cells of normal rat aorta and more importantly after balloon injury.19 Moreover, expression of mRNA for angiotensinogen and ACE has been demonstrated in different arterial vessels, such as rat aorta (smooth muscle and adventitia) and mesentery.20–24 Debate still remains25 regarding the capacity of the vascular tissue to synthesize renin, since molecular biological techniques have demonstrated only low levels of renin mRNA,23,26 and in some studies mRNA renin could not be detected at all.27 However, the low mRNA level for renin does not necessarily exclude the existence of a functional local RAS,25 since the uptake of renin by vascular tissue is avid26–31 and the presence of renin-like enzymes in human vasculature has been demonstrated.32

Finally, different metabolism and production of Ang I in different organs and vascular beds have been reported in patients with hypertension.33

Taken together, these data suggest the hypothesis that a RAS exists in the vasculature. To investigate this hypothesis, we combined the measurement of arterial-venous gradient of the endogenous Ang I and Ang II in two different vascular beds.
Selected Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
Ang I, II = angiotensin I, II
HPLC = high-performance liquid chromatography
PRA = plasma renin activity
RAS = renin-angiotensin system

beds (forearm and leg) with the kinetics of 125I-Ang I and 125I-Ang II in different functional conditions. Highly purified monoidinate 125I-Ang I and 125I-Ang II have been demonstrated to be appropriate for measurements of Ang I to Ang II conversion and Ang I and Ang II degradation14-20 because the radiolabeled angiotensins have been found to have the same plasmatic clearance as the endogenous angiotensins.33,36

Methods

Subjects Investigated

Twenty-eight normotensive healthy subjects (19 men and 9 women; 36±5 years old, [range, 25 to 43]), were investigated. Entry criteria included no medication use for at least 15 days before the test and no history of angina, myocardial infarction, stroke, diabetes, and hypertension. Entry laboratory requirements included normal levels of hematocrit, fasting blood glucose, and total cholesterol and normal findings on resting electrocardiogram. All subjects gave written consent after a full explanation of the purposes and potential risks involved in participating in the study. The protocol of this study complies with the principles of the Helsinki declaration of the World Medical Association.37

Study Design

Ang I and Ang II were measured in all the subjects in peripheral arterial (radial or brachial artery) and venous (antecubital vein) blood of the forearm; in 20 subjects additional blood samples were withdrawn from the femoral vein to investigate the kinetics and production rate of Ang I and Ang II in the leg vascular bed also. PRA was assayed in peripheral venous blood. All patients were investigated after 1 week of a normal sodium diet (108 mmol/d). Because it is possible to calculate 125I-Ang II extraction when the arterial and venous 125I-Ang I and II levels during 125I-Ang I infusion are known,36 to minimize the total radioactivity for each patient, 125I-Ang I and 125I-Ang II infusions were not performed in the same subjects but were studied in two groups of subjects. Specifically, we studied 125I-Ang I kinetics in 14 patients (group A) and 125I-Ang II kinetics in the remaining 14 patients (group B). Successively, the patients of each group were randomly assigned: 7 to low sodium intake (20 mmol/d) and 7 to high sodium intake (108 mmol/d) to study 125I-Ang I and 125I-Ang II kinetics in the remaining 14 patients. Five subjects for 125I-Ang I and 10 for 125I-Ang II, respectively. Five subjects for each group were randomly assigned to low or high sodium diet.

Experimental Procedure

All the subjects were studied in the morning and in an overnight fasting state. Tea, coffee, and alcohol were withheld for a minimum of 24 hours before the study. Ten of 28 subjects were smokers, but all abstained from smoking during the 24 hours preceding the study. On the day of the angiotensin infusion, subjects remained in a supine position in an air-conditioned room (room temperature of about 22°C to 23°C) for 2 hours before the test was performed. With the subject under local anesthesia with 2% procaine, the left brachial artery was cannulated with an 18-gauge intravascular catheter (Inpharden cath-
eter, Inphardial SpA,) for collection of the arterial samples; 18-gauge intravascular catheters were inserted into the left antecubital vein for sampling of venous blood and into the right antecubital vein for 125I-Ang I or 125I-Ang II infusion. In the 20 subjects in whom kinetics of radiolabeled angiotensin was also studied in the leg vascular region, the right femoral vein was cannulated with an 18-gauge intravascular cannula. After 30 minutes of supine rest and 15 minutes after the insertion of catheters, 125I-Ang I or 125I-Ang II (specific radioactivity, 81.4 TBq/mmol) was infused at a rate of approximately 3.5-6.25 MBq/kg for 20 minutes. Both arterial and venous levels of 125I-Ang I or 125I-Ang II reached a plateau and were constant in the period between 10 and 15 minutes after the start of the infusion (coefficient of variation <5%).31 In this period, blood samples for endogenous and radiolabeled Ang I and Ang II assays were contemporaneously drawn from the brachial artery and the antecubital vein in all subjects; in 20 subjects additional samples were taken from the femoral artery.

Soon after blood sampling, forearm (n=28) and leg (n=20) blood flow were measured by venous-occlusion strain-gauge plethysmography (Periflow SU4 Jansen Instruments). Arterial occlusion to the hand or foot was provided by a cuff connected via a three-way tap to a reservoir containing air at a pressure of 200 mm Hg. Each measurement was automatically made by the plethysmograph, synchronized with heart rate, so as to occlude the veins during 3 heart beats and to release them during 2; each value was then obtained from the average of 5 consecutive measurements. Forearm blood flow was usually measured at the right arm, while blood pressure was measured with a mercury sphygmomanometer on the opposite arm. The patients were continuously monitored with electrocardiography. Limb vascular resistance was calculated from the ratio between mean blood pressure and forearm or calf blood flow.

Assay of Ang I and Ang II

Blood for angiotensin assays was rapidly drawn with a plastic 5-mL syringe containing 0.5 mL inhibitor solution (0.125 mmol/L disodium EDTA, 0.025 mol/L 1,10-phenanthroline, 2 mg/mL neomycin, 1 mg/mL captopril, and the renin inhibitor Ro-42–5892, kindly provided by Dr Walter Fischli, La Roche, Basel, Switzerland)39,40 and was transferred into prechilled plastic tubes. Blood samples were centrifuged at 2000g for 20 minutes at 4°C and stored at −20°C until analyzed. Blood for PRA measurements (7 mL) was collected into 10-mL plastic tubes containing 0.2 mL disodium EDTA (final concentration, 5 mmol/L). Blood samples were centrifuged at 2000g for 20 minutes at 4°C and stored at −20°C until analyzed.

Angiotensin assay was performed as previously described in detail.40 Briefly, Ang I and II were extracted from plasma using Sep-Pak C-18 cartridges (Waters Associates) and were separated by reverse-phase HPLC (6000 A Waters Pump, Waters Spa, equipped with a Nucleosil C-18 steel column, 250×4.6 mm, 10-μm particle size, Perkin-Elmer). Elution was performed as follows: 65% of 0.085% orthophosphoric acid containing 0.02% sodium azide (pH 2.33) (mobile phase A)/35% methanol (mobile phase B) from 0 to 9 minutes followed by a linear gradient to 40% A/60% B until 23 minutes was reached. The flow was 1 mL/min, and the working temperature was 45°C. Eluate was collected in 1-minute fractions into polyethylene tubes and evaporated in the concentrator before radioimmunoassay, after which pH value of blood samples was corrected to 7.4.

The extraction and HPLC yielded a good separation among Ang I, Ang II, and their metabolites, with high reproducibility of retention times (variation coefficient of 1.2% and 1.7% for injection-to-injection and day-to-day variability, respectively), which allowed the recovery of Ang I and Ang II in picomolar quantities from plasma extract in fractions corresponding to Ang I and Ang II peaks. Similarly, HPLC separation patterns for radiolabeled angiotensin plasma extracts demonstrated a clear separation between the peak of 125I-Ang I and 125I-Ang II and other metabolites. The retention times of 125I-Ang II and 125I-Ang I were 12 and 21 minutes, respectively.

The extraction recovery of unlabed angiotensins was determined by adding 35 fmol of (Ile5)-Ang I and (Ile5)-Ang II to 6 plasma samples: the overall recovery was 96.7±6% for (Ile5)-Ang I and 97.8±5.4% for (Ile5)-Ang II. The extraction recovery of radiolabeled angiotensins was determined by adding 125I-Ang I and 125I-Ang II (6000 cpm for each peptide) to 1-mL portions of
Human Vascular Renin–Angiotensin System

Characteristics of Radiolabeled Ang I and Ang II

125I-Ang I and 125I-Ang II were obtained from Du Pont de Nemours, NEN Division. Immediately before each infusion, 125I-Ang I or 125I-Ang II solution was sterilized by filtration through a 0.22-mM Millipore membrane filter (Waters). The specific radioactivity of the 125I-Ang I and 125I-Ang II preparations was 81.4 TBq/mmol. The purity of radiolabeled solutions assessed by the injection of a sample into the HPLC column and by the count of the collected fractions was >99%. The elimination half-life of 125I-Ang I and of 125I-Ang II, measured in 6 subjects in a preliminary phase of the study, was 0.72 ± 0.17 minute, which is similar to the elimination half-life (0.70 ± 0.23 minute) of the unlabeled angiotensins (Ang I and Ang II, Sigma Chemical Co) that were contemporaneously infused in the same subjects at a rate of approximately 1.5 ng/min for 20 minutes.

Urinary excretion of radioactivity was followed for a period of 96 hours. Of the administered radioactivity, 88% was excreted within 24 hours and 98% was recovered in the 96-hour period. The calculated exposure to radioactivity was 0.24 μGy (or 0.6 mrad) from 125I-Ang I or Ang II infusion.

125I-Ang I and 125I-Ang II were extracted from plasma according to the procedure described for the noniodinated peptides, and their concentrations in collected chromatographic fractions were measured directly in a 12-channel gamma counter (Multigamma 1261 LBK-Wallak, EG & G) for 20 minutes.

All subjects received 5 mL/d Lugol’s solution from 2 days before to 4 days after the 125I-Ang I or 125I-Ang II infusion.

Parameters for Evaluation of Ang I and Ang II Kinetics

For the evaluation of 125I-Ang I and 125I-Ang II kinetics, the following parameters were used as suggested by Admiral et al.38 The validity and the reliability of these parameters were confirmed in previous studies.39,40

For 125I-Ang I kinetics, (1A) regional extraction (conversion plus degradation) = 1 – (125I-Ang II/125I-Ang I); (1B) venous Ang I derived from arterial delivered Ang I = arterial blood Ang I × (1 – regional extraction); (1C) venous Ang I formed by circulating PRA during regional passage = PRA × arterial blood transit time (Regional blood transit times are from the literature.39,40); (1D) venous Ang I derived from the forearm or leg passage (de novo regional Ang I) = Ang II venous – (Ang I venous × [1 – regional extraction]); (1E) venous Ang I derived from regional tissue production (de novo Ang I) = (Ang I formed by PRA); (1F) formation of Ang I and Ang II in relation to regional blood flow (output) = pg/mL × mL/min = pg/min; (1G) fractional Ang I conversion rate = 125I-Ang I extraction × k12t/ k11t + k21t, fractional degradation of Ang I = 125I-Ang I extraction × k11t/k11t + k21t.

According to the venous equilibrium model described by Danser et al.,41 k11t is the first-order constant for degradation of Ang I and Ang II to other metabolites and k21t is the first-order constant for conversion of Ang I to Ang II.

For 125I-Ang II kinetics, (2A) regional extraction = 1 – (125I-Ang II/125I-Ang II venous); (2B) 125I-Ang II extraction can be calculated from the results obtained during infusion of 125I-Ang I alone as k1t = –ln(1 – 125I-Ang II extraction); (2C) regional Ang II derived from arterial delivered Ang II = arterial blood Ang II × (1 – regional extraction); (2D) venous Ang II derived from de novo regional formation = Ang II venous – (Ang II venous × [1 – regional extraction]); (2E) venous Ang II derived from the conversion of arterially delivered Ang I: arterially delivered Ang I × (125I-Ang I extraction – 125I-Ang II extraction).

Statistical Analysis

Data are presented as mean±1 SD. A paired Student’s t test was used to compare Ang I and Ang II metabolism parameters found during normal sodium intake with those measured during low or high sodium intake. One-way ANOVA was used to assess differences in parameters of 125I-Ang I and 125I-Ang II kinetics among different sodium diets. Values are considered significantly different at a value of P<.05.

Results

Angiotensin Formation in Forearm Vascular Bed With Normal Sodium Intake

In the 28 subjects investigated, PRA in venous blood was 10.5 ± 2.5 pmol · L−1 · min−1 (range, 4.5 to 15.2 pmol · L−1 · min−1). Ang I concentration had a mean value of 14.9 ± 4.4 pmol/L in arterial blood and 16.7 ± 4.6 pmol/L in venous blood, resulting in a arterial-venous gradient of 1.8 ± 2.1 pmol/L.

Forearm blood flow was on average 2.9 ± 0.2 mL · 100 mL−1 · min−1, and because mean blood pressure was 92.8 ± 3.9 mm Hg, peripheral vascular resistance was 32.2 ± 2.6 U. Forearm Ang I arterial-venous gradient, when related to forearm blood flow, was on average 5.7 ± 4.1 pmol · 100 mL−1 · min−1. Ang II concentration was on average 7.1 ± 2.2 pmol/L in arterial blood and 8.1 ± 2.5 pmol/L in venous blood, with an arterial-venous gradient of 0.8 ± 1.5 pmol/L and a forearm output of 2.5 ± 4.6 fmol · 100 mL−1 · min−1.

The arterial and venous Ang I and Ang II concentrations in subjects of group A were not significantly different from the values found in the subjects of group B (always P>0.05).

Kinetics of 125I-Ang I was investigated in 14 subjects (group A). Steady state 125I-Ang I and 125I-Ang II levels are shown in Table 1. In all subjects 125I-Ang I was extracted during the forearm arterial-venous passage, with a mean extraction of 35.7±6.1%. As a consequence, Ang I delivered arterially to venous blood was 8.5 ± 2.6 pmol/L, significantly lower than the actual Ang I concentration found in venous blood (16.7 ± 4.6 pmol/L). This finding indicates that during the passage through the forearm, on average 6.5 ± 2.2 pmol/L of Ang I was added to venous blood. Specifically, 1.2 ± 0.7 pmol/L was formed by PRA and 5 ± 2.2 pmol/L was added by forearm vascular tissue, with a regional output of 14.9 ± 5.1 pmol · 100 mL−1 · min−1 (Table 1). The fractional conversion rate of 125I-Ang I to 125I-Ang II during the passage of blood across the forearm vascular bed was 12 ± 4% (Table 1). From the 125I-Ang I kinetics study, the extraction of 125I-Ang II was calculated according to formula 2B in “Methods” and was found to be 25.9 ± 2.4%.

Kinetics of 125I-Ang II was studied in the 14 subjects who had not been randomly allocated to the study of 125I-Ang I kinetics (group B). Steady state 125I-Ang II levels are shown in Table 1. Extraction of 125I-Ang II during the passage of blood through the forearm vascular tissue was on average 29.7 ± 5.1%. This value was not significantly different from the extraction value of 125I-Ang II calculated in the subjects of group A on the basis of the data obtained during 125I-Ang I kinetics. By considering the extraction of 125I-Ang II, the
amount of Ang II present in venous blood should have theoretically been 4.9 pmol/L, a value significantly lower than the Ang II concentration (8.1±2.5 pmol/L) actually found in venous blood. This finding indicates that on average 2.8±1.4 pmol/L of Ang II was added to blood during the passage across the forearm vascular bed. Because the extraction of 125I-Ang II, as well as the arterial and venous concentrations of Ang I and Ang II, was not significantly different between group A and group B, the amount of Ang II derived from the conversion of arterially delivered Ang I (0.6±0.5 pmol/L) was used to calculate the amount of Ang II formed by vascular tissue. This amount was found to be 2.1±0.9 pmol/L (Table 1, Fig 1), a value not significantly different from the amount of Ang II formed by vascular tissue in subjects of group A (2.3±0.8 pmol/L). Therefore, forearm vascular tissue produces on average 6.2±2.8 fmol · 100 mL⁻¹ · min⁻¹ of Ang II.

**Effects of Low Sodium Diet on Ang I and Ang II Extraction and Formation by Forearm Vascular Bed**

Seven of 14 subjects from group A (125I-Ang I kinetics in normal sodium diet) and 7 of the 14 subjects from the group B (125I-Ang II kinetics in normal sodium intake) were randomly allocated to low sodium intake. After a week of low sodium diet, PRA significantly increased from 10.5±1.5 to 46.6±12.6 pmol · L⁻¹ · min⁻¹ (P<.001), and forearm blood flow slightly decreased in all 14 subjects, passing from 2.9±0.2 to 2.6±0.7 mL · 100 mL⁻¹ · min⁻¹ (r=1.9 NS). Mean blood pressure did not significantly change (from 92.8±3.9 to 93.6±5.8 mm Hg), whereas forearm vascular resistance increased from 32.1±2.5 to 33.9±2.6 UI (P<.03).

Although Ang I and Ang II concentrations increased both in arterial and venous blood to 25.1±4.6 and 19.3±3.8 pmol/L for Ang I and to 17.3±3.1 and 11.8±2.8 pmol/L for Ang II (all P<.01 versus baseline), arterial-venous forearm gradient became markedly negative (−7±3.3 pmol/L for Ang I and −5.9±1.1 pmol/L for Ang II; P<.01 versus baseline). The modification of sodium intake notably changed Ang I and Ang II formation by vascular tissue (Table 2, Fig 1). Extraction of 125I-Ang II mildly decreased (from 36±6% to 33±7%, P<.05), whereas extraction of 125I-Ang II did not change. Ang I formed by PRA during the passage of blood across the forearm vascular bed was considerably augmented (from 1.4±2.2 to 8±4.9 pmol/L, P<.01) (Fig 1), and the Ang I arterially delivered to venous blood was significantly increased (from 8.5±2.6 to 15.7±5.1 pmol/L, P<.01). Conversely, the amount of Ang II added during the blood passage across forearm vascular bed markedly decreased (from 6.5±2.2 to 1.9±1.7 pmol/L, P<.001), and Ang II production by vasculature became undetectable (Table 2). Also, the conversion of 125I-Ang I to 125I-Ang II significantly decreased from 12±4 to 6±1% (P<.01). The amount of Ang II added to blood during the passage across forearm vascular bed significantly decreased (from 2.8±1.4 to 0.7±1.2 pmol/L, P<.01), and the study of 125I-Ang I kinetics indicated that added Ang II derived completely from arterially delivered Ang I (Table 2, Fig 1).

**Effects of High Sodium Diet on Extraction and Formation of Ang I and Ang II by Forearm Vascular Bed**

Fourteen patients for whom 125I-Ang I (n=7) or 125I-Ang II (n=7) kinetics were performed with a normal sodium diet and had not been on a low sodium diet were randomly allocated to...
Changes in sodium intake significantly modified $^{125}$I-Ang I (panels A and B) and $^{125}$I-Ang II (panels C and D) metabolism through forearm tissue. The changes in tissue formation of both Ang I and Ang II (panels B and D) were opposite to those of circulating RAS activity. With low sodium diet (B), circulating RAS was markedly activated and both Ang I and Ang II formation by forearm vascular tissue was significantly decreased ($P<.01$ vs normal sodium intake), whereas with high sodium intake (D) circulating RAS was depressed and tissue formation of angiotensins significantly increased ($P<.01$ vs normal sodium diet). Ang II formation by Ang I to Ang II conversion was slightly modified both with low and high sodium intake despite significant changes in Ang I to Ang II fractional conversion rate (both $P<.01$ vs baseline).

High sodium intake. At the end of the period on a high sodium diet, mean blood pressure did not change significantly (from $91.6\pm5.1$ to $92.8\pm4.7$ mm Hg), and forearm vascular resistance decreased slightly but not significantly (from $31.6\pm2.5$ to $30.7\pm0.9$ UI). PRA and Ang I concentrations in arterial and venous blood were significantly lower than with normal sodium intake ($7.2\pm3.5$ versus $10.5\pm1.5$ pmol/L/min and $10.4\pm3$ versus $13.7\pm3$ pmol/L, respectively; $P<.01$ vs baseline). Ang II levels in arterial and venous blood changed slightly, but arterial-venous gradient of both Ang I and Ang II was more positive than that found with normal sodium diet subjects ($3.7\pm1.5$ and $4.6\pm1$ pmol/L versus $1.7\pm1.2$ and $1\pm1.3$ pmol/L, respectively; $P<.001$ for both). These changes occurred despite a significantly increased vascular extraction of both $^{125}$I-Ang I and $^{125}$I-Ang II and a notable decrease in Ang I formation by PRA during the passage of blood across the forearm vascular bed (Fig 1). The addition of Ang I to blood during the passage across the forearm vascular bed was increased (from $7.3\pm2.5$ to $9.5\pm2.4$ pmol/L, $P<.01$). The increase was essentially due to the Ang I added by vascular tissue (Table 2). Accordingly, the addition of Ang II during the passage of blood across the vascular bed was increased (from $2.8\pm1.4$ to $4.9\pm1.2$ pmol/L, $P<.001$), and the $^{125}$I-Ang I kinetics indicated that only $0.9$ pmol/L of the added Ang II derived from the conversion of arterially delivered Ang I (Fig 1) and then $3.9\pm1.5$ pmol/L of Ang II were formed by vascular tissue (Table 2).

**TABLE 2. Changes in De Novo Ang I and Ang II Formation by Forearm Vascular Tissue Induced by Modifications in Sodium Intake**

<table>
<thead>
<tr>
<th></th>
<th>Low Sodium Diet</th>
<th>Normal Sodium Diet</th>
<th>High Sodium Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Ang I formation, pmol/L</td>
<td>Undetectable</td>
<td>$5.1\pm2.2$</td>
<td>$7.8\pm2.3^*$</td>
</tr>
<tr>
<td>Tissue Ang II formation, pmol/L</td>
<td>Undetectable</td>
<td>$2.1\pm0.9$</td>
<td>$3.9\pm1.5^*$</td>
</tr>
</tbody>
</table>

Data represent mean$\pm$SD. Tissue Ang I formation was measured in 14 subjects successively allocated to low (n=7) or high (n=7) sodium diet. Tissue Ang II formation was calculated in 28 subjects successively allocated to low (n=14) or high (n=14) sodium diet.

$^*$ $P<.01$ vs normal sodium diet.

**Metabolism and Formation of Ang I and Ang II by Leg Vascular Bed**

Kinetics of radiolabeled angiotensins and formation of both Ang I and Ang II during the passage of blood across the leg vascular bed were not substantially different from those of the forearm vascular bed (Table 1). Arterial-femoral vein gradients of both Ang I and Ang II were slight positive, similarly to those observed in the forearm. Extraction of $^{125}$I-Ang I and of $^{125}$I-Ang II by leg vascular tissue was slightly but significantly higher ($P<.01$ and $P<.05$, respectively) than the extraction found in the forearm vascular tissue (Table 1). Conversion of $^{125}$I-Ang I to $^{125}$I-Ang II was not different between the two vascular beds. Even during the passage of blood across the leg vascular bed, both Ang I and Ang II were added in an amount similar to those found for forearm vascular tissue (Table 1).

Both low and high sodium intakes significantly changed the amount of both Ang I and Ang II added to blood during the passage across vascular bed. These modifications paralleled those observed in the forearm vascular bed during the changes of sodium intake.

**Discussion**

The present results provide evidence for a functional local RAS in the vascular tissue of humans. By combining $^{125}$I-Ang I and $^{125}$I-Ang II kinetics with the measurements in plasma of endogenous angiotensins under different conditions of sodium intake, we were able to demonstrate a different local formation of both Ang I and Ang II related to the different experimental settings.

Artifacts and nonspecific measurements of Ang I and Ang II may lead to incorrect measurements of the real angiotensin formation. However, HPLC separation of Ang I and Ang II caused formation of very distinct peaks of the two angiotensins and their metabolites, and subsequently a very sensitive radioimmunoassay was used. In addition, $^{125}$I-Ang I and $^{125}$I-Ang II were well differentiated by HPLC, and the retention times of $^{125}$I-Ang I and $^{125}$I-Ang II differed from the retention times of other angiotensin peptides. Thus, the methods used seem adequate for the aim of the present study.

During the passage through both forearm and leg vascular beds, Ang I underwent important metabolic changes. Approx-
imately 35% and 37% of the Ang I passing across the two vascular beds were extracted, partly converted to Ang II, and partly degraded by angiotensinases into smaller inactive peptides. Despite this extraction, Ang I concentration in reffuent venous blood from forearm or leg vascular beds was slightly but significantly higher than in the respective nutrient arteries, thus indicating that Ang I is formed during the passage across these vascular beds. Because Ang I formation by PRA during this passage (for an average transit time of 7 to 11 seconds) was very low (<1.8 pmol/L) for both vascular beds, i.e., <10% of the total amount of Ang I present in the venous blood, the remaining amount of Ang I present in venous blood after extraction has to be produced de novo during the passage through the vascular bed. Ang I formation by PRA in the intravascular cannula and in the tube appears negligible because the transit time of blood from the artery or the vein to the syringe was about 1 second, and in the syringe a potent renin inhibitor was present. Therefore, the amount of Ang I found in the reffuent venous blood from forearm or leg vascular beds, exceeding the sum of the arterially delivered Ang I plus Ang I formed by PRA, was added to by vascular tissue.

Ang II is also formed during the passage of blood across the vascular beds of the forearm and the leg, as is indicated by the slightly positive arterial-venous gradient despite the 30% extraction of the arterially delivered 125I-Ang II. Arterially delivered Ang II (4.9 pmol/L for the forearm and 4.7 pmol/L for the leg vascular bed) plus Ang II derived from conversion of arterially delivered Ang I (0.6 pmol/L) is significantly less than the total concentration of Ang II in the venous blood (8.1 pmol/L for the forearm and 7.8 pmol/L for the leg). Thus, about 2.1 and 2.2 pmol/L of Ang II were added by forearm and leg vascular beds, respectively.

We did not examine the pathways of Ang II formation by vascular tissue; therefore, we cannot exclude that enzymes other than ACE, such as the chymostatin-sensitive Ang II generating enzyme or peptidases, may be involved. However, in human intact heart and in solubilized left ventricular membrane preparations from explanted failing hearts, the largely predominant pathway for Ang II formation is through ACE. No relevant difference seems to exist between the RAS of the forearm vascular tree and that of the leg vascular tree, except for a slightly higher Ang II formation in the leg vascular bed when related to the blood flow (11.1 ± 5.5 fmol/100 mL/min compared with 8.1 ± 3.4 fmol/100 mL/min of the forearm vascular bed).

In a recent study performed in patients with essential hypertension and with unilateral renal artery stenosis and elevated PRA, no evidence was found for a source of circulating Ang II in reffuent venous blood from forearm and leg vascular bed other than blood-borne Ang I. However, in this investigation we studied only hypertensive patients whose PRA was on average about twofold (31 pmol · L⁻¹ · min⁻¹ in patients with essential hypertension and 21 pmol · L⁻¹ · min⁻¹ in patients with unilateral renal artery stenosis) that of the normotensive healthy subjects investigated in our study (10 pmol · L⁻¹ · min⁻¹). Therefore, the condition of hypertensive patients is similar to the activation of PRA present with low sodium diet, which in normal subjects is associated with the incapacity to detect formation of Ang II by vascular tissue.

The vascular RAS showed important functional changes in relation to different sodium intakes. These modifications involved both Ang I formation and ACE activity, with consequent different Ang II generation. The functional changes of the vascular RAS were opposite to those of circulating PRA so that when PRA is increased as with low sodium diet, vascular ACE activity and vascular Ang II generation were reduced, and the opposite occurred when PRA was depressed as with high sodium intake. These findings indicate that changes in the vascular renin activity of vascular RAS are not due to a passive phenomenon but are the result of a finely regulated mechanism(s) proposed to maintain the homeostasis between the local and systemic formation of Ang II.

Even if the amount of Ang II formed by vasculature is only a few picomoles per liter (from undetectable in low sodium diet to 8.2 pmol/L in high sodium intake, compared with 2.8 pmol/L in normal sodium diet for the forearm vascular bed), the differences in Ang II formation are from 3 to 20 times greater than interassay and intra-assay variability; most importantly, the changes in Ang II generation by vasculature following modifications of sodium intakes were homogeneous in all the subjects. It is worth stressing that the concentration of Ang II in venous blood represents the amount of Ang II that has not bound to high-affinity Ang II receptors of vascular tissue. Therefore the amount of Ang II formed by the vasculature is not at all negligible when related to the total mass of the vascular beds.

All the layers of the vascular wall may contribute to the vascular tissue–based formation of the angiotensins. Ang I may be locally synthesized by the action of renin on angiotensinogen, which may be taken from plasma or locally produced in the medial and adventitial layers, where mRNA for renin and angiotensinogen has been demonstrated. Conver-
sion of Ang I to Ang II may occur both in the endothelium and/or in the medial layer, which are provided with mRNA for ACE. Thus, the formation of Ang II in vascular beds can occur both in vessels provided with medial and adventitia layers and in the microvasculature.

The vascular RAS has functional characteristics similar to those of the cardiac RAS, i.e., tissue-based Ang I and Ang II generation and important changes in local angiotensin formation in relation to different sodium intakes. In addition to this autocrine-paracrine activity, vascular RAS seems to participate in the general homeostasis of Ang I and Ang II, both through the degradation of angiotensins and through the local formation of both Ang I and Ang II. About 30% of Ang I and Ang II were degraded by angiotensinases, and only 11% to 12% of Ang I was converted to Ang II during the passage of blood through vascular bed. Likewise, the changes in Ang I and Ang II formation by vascular tissue, contrary to changes in plasma Ang II concentrations as observed with different sodium intakes, seem to indicate that vascular RAS may operate as a feedback mechanism in relation to the arterial Ang II concentration.

In conclusion, the present results provide consistent evidence for the existence in humans of a functional vascular RAS independent from but related to the circulating RAS. The existence of a vascular RAS may have relevant pathophysiological and clinical implications because an altered function of
the local system may not result in a systemic blood pressure effect but may be implicated in local processes such as vascular hypertrophy and remodeling, atherosclerosis, and restenosis.

References
Human Vascular Renin-Angiotensin System and Its Functional Changes in Relation to Different Sodium Intakes
Maria Boddi, Loredana Poggesi, Mirella Coppo, Nicoletta Zarone, Simona Sacchi, Chechi Tania and Gian Gastone Neri Serneri

Hypertension. 1998;31:836-842
doi: 10.1161/01.HYP.31.3.836

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 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/31/3/836

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/