Renal Interstitial Fluid Concentrations of Angiotensins I and II in Anesthetized Rats

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Abstract—Previous studies have indicated that angiotensin II (Ang II) concentrations in renal interstitial fluid are much higher than plasma levels. In the present study, we performed experiments to explore renal interstitial fluid concentrations of Ang I and Ang II further and to determine whether these levels are altered by acute arterial infusion of an ACE inhibitor (enalaprilat) or by volume expansion. Microdialysis probes (molecular weight cutoff point: 30 000 Da) were implanted in the renal cortex of anesthetized rats and were perfused at a rate of 2 μL/min. Using relative equilibrium rates, the basal renal interstitial fluid Ang II concentration averaged 3.07±0.43 nmol/L, a value much higher than the plasma Ang II concentration of 107±8 pmol/L (n=7). Interstitial fluid Ang I concentrations (0.84±0.04 nmol/L) were consistently lower than the Ang II concentrations but higher than the plasma Ang I concentrations (112±14 pmol/L). Intra-arterial infusion of enalaprilat (7.5 μmol/kg/min, n=5) for 120 minutes resulted in a significant decrease in mean arterial pressure (from 114±4 to 68±4 mm Hg) along with reductions in plasma and renal ACE activity (by −99% and −52%, respectively). Enalaprilat resulted in a significant increase in plasma Ang I from 133±21 to 1167±328 pmol/L and a decrease in plasma Ang II from 110±12 to 67±9 pmol/L. During enalaprilat infusion, interstitial fluid concentration of Ang I was significantly increased from 0.78±0.06 to 0.97±0.08 nmol/L; however, Ang II concentrations were not altered significantly (3.67±0.28 versus 3.67±0.25 nmol/L). Acute volume loading with Ringer’s solution containing 1% bovine serum albumin at a rate of 150 μL/min for 2 hours (6% to 7% of body weight) lowered plasma concentrations of Ang I from 110±23 to 16±2 pmol/L and Ang II from 100±23 to 36±6 pmol/L; however, renal interstitial fluid concentrations of Ang I and Ang II were not altered significantly during volume expansion (Ang I, from 0.77±0.05 to 0.69±0.03 nmol/L; Ang II, from 3.76±0.43 to 3.59±0.39 nmol/L, n=5). These data indicate that renal interstitial fluid concentrations of Ang I and Ang II are substantially higher than the corresponding plasma concentrations. Furthermore, the fact that the high interstitial fluid concentrations of Ang II are not responsive to acute ACE inhibition or volume expansion suggests the compartmentalization and independent regulation of renal interstitial fluid Ang II. (Hypertension. 2002;39:129-134.)

Key Words: angiotensin II ● angiotensin I ● kidney ● angiotensin-converting enzyme inhibitor

Angiotensin II (Ang II) exerts a critical role in the paracrine regulation of renal function and the pathophysiology of hypertension.1–5 Intrarenal Ang II is formed locally, as evidenced by the fact that kidney tissue Ang II contents are much greater than can be explained solely on the basis of equilibration with circulating concentrations of Ang II.6–9 It has also been shown that the kidney contains all the necessary components to generate Ang II from its precursor angiotensinogen10,11 and that intrarenal Ang II formation can be altered independently of the plasma levels in response to various maneuvers.6–8,12 The findings that angiotensinogen10,13 and angiotensinogen mRNA14,15 are localized in the proximal tubule cells suggest either that proximal tubules produce Ang II subsequent to angiotensinogen secretion or that Ang II may be formed intracellularly. Indeed, micropuncture studies have demonstrated that proximal tubular fluid concentrations of Ang I and Ang II are in the nanomolar range and are much higher than plasma concentrations.12,16–18

These observations indicate that proximal tubule cells are the source of the intratubular Ang II. It has also been suggested that renal interstitial fluid contains high concentrations of Ang II.19–23 Mitchell and Navar24,25 showed that the direct perfusion of Ang I and Ang II into the peritubular capillaries results in afferent arteriolar vasoconstriction, decreased single nephron glomerular filtration rate, and increases in proximal fractional reabsorption rate. Although these results indicate that substantial diffusion of the peptides from the peritubular capillaries into the interstitium can occur, the mechanisms that regulate the renal interstitial fluid Ang I and Ang II concentrations have remained unresolved.

Using immunocytochemistry, it has been shown that juxtaglomerular cells contain angiotensinogen, renin, Ang I, and...
Ang II. These data suggest that juxta-glomerular cells produce Ang II and release it into the renal interstitium. Results from measurements of Ang II in renal lymph showed that Ang II concentrations in renal lymph are much higher than those in arterial or renal venous plasma. Although it is likely that some Ang II generation occurs during the transit of lymph fluid to the collection site, high levels of Ang II in renal lymph are consistent with the notion that substantial Ang II is formed within the renal interstitium or added to the interstitial compartment. As an alternative means of assessing renal cortical interstitial Ang II levels, Siragy et al implanted microdialysis probes that had a molecular weight cutoff of 5000 Da, thus allowing equilibration of smaller molecules and the angiotensin peptides. The authors showed that the renal interstitial fluid Ang II concentrations in dogs and rats are much higher than the plasma levels. With this technique, various enzymes with higher molecular weights would not pass through the membrane, and thus, there should be limited formation and/or metabolism of Ang II once it diffuses across the dialysis membrane. Collectively, these studies suggest that the renal interstitial fluid concentrations are regulated independently of the circulating Ang II concentrations. To address this issue further, it is necessary to assess renal interstitial fluid concentrations of Ang I, as well as total kidney Ang I and Ang II contents in the same animals.

The current in vivo microdialysis experiments were designed to explore further the presence of Ang I and Ang II in renal interstitial fluid. To exclude the possible in vitro generation or degradation of Ang II, dialysate fluid was directly collected from the outflow steel tubing of microdialysis probes. The tip of the outflow steel tubing was placed into a solution of inhibitors, so that dialysate effluent could be immediately mixed with the inhibitors. In addition, arterial blood and the kidneys in which the microdialysis probes were implanted were harvested after completion of the microdialysis procedures. These results allowed us to compare renal interstitial fluid Ang I and Ang II concentrations with the total tissue levels from the same kidneys. Studies were also performed to determine whether the renal interstitial peptide levels respond differently to acute arterial infusion of an ACE inhibitor (enalaprilat) or volume expansion compared with changes in plasma Ang I and Ang II.

Methods

Animal Preparation

The experiments were performed in accordance with the guidelines and practices established by the Tulane University Animal Care and Use Committee. Sprague-Dawley rats, weighing 265 to 330 g, were anesthetized with Inactin (100 to 120 mg/kg, IP). The surgical preparation of the animals and basic experimental techniques were identical to those previously described. During surgical preparation, Ringer’s solution containing 6% BSA (pH=7.4) was infused intravenously at a rate of 20 µL/min to replace volume losses. Thereafter, Ringer’s solution containing 1% BSA (pH=7.4) was infused at a rate of 20 µL/min for the duration of the stabilization and experimental periods.

Characteristics of the Microdialysis Probe

For the determination of renal interstitial concentrations of Ang I and Ang II, we used an in vivo microdialysis method using probes with a 30,000 Da cutoff. Further details of the microdialysis method are available in the expanded Methods section presented online at http://www.hypertensionaha.org.

Effects of Intra-Arterial Infusion of Enalprilat on Renal Interstitial Fluid Ang I and Ang II

The experimental protocol was started with dialysate fluid collections for 2 consecutive 30-minute periods. At the end of the second control collection, an arterial blood sample was taken from a catheter placed in the left carotid artery. Then, enalaprilat was infused intra-arterially from a femoral catheter placed in the aorta just above the origin of the left renal artery at a rate of 0.2, 1, and 7.5 µmol/kg/min (n=6, 8, and 5, respectively). Enalaprilat was dissolved in Ringer’s solution containing 1% BSA (pH=7.4) and administered at 10 to 15 µL/min. The intravenous infusion of Ringer’s solution containing 1% BSA was adjusted so that the total volume infused was kept at 20 µL/min. After 30 minutes of enalaprilat infusion, 4 consecutive 30-minute dialysate samples were collected. At the end of each experiment, an arterial blood sample was taken and the kidneys in which microdialysis probes were implanted were harvested. Ang I and Ang II concentrations in plasma and ACE activity in plasma and renal tissue were measured.

Effects of Acute Volume Expansion on Renal Interstitial Fluid Ang I and Ang II

Experiments were performed to determine whether interstitial concentrations of Ang I and Ang II are influenced by changes in plasma concentrations elicited by acute volume expansion (n=5). After the control periods, the intravenous infusion rate of Ringer’s solution containing 1% BSA of 20 µL/min was increased to 150 µL/min. Thirty minutes after increasing the infusion rate, 3 consecutive 30-minute dialysate samples were collected. This infusion rate was selected to cause a volume expansion of approximately 6% to 7% of body weight after 2 hours of infusion.

Analytical Procedures

Plasma and renal tissue Ang I and II levels were measured by radioimmunoassay, as previously described. The dialysate samples, collected in 100% methanol, were evaporated to dryness in a vacuum centrifuge, reconstituted in assay buffer, and assayed. ACE activity was measured by fluorometric measurement of the enzymatic cleavage of hippurate from hippuryl-histidyl-leucine, as previously reported.

Statistical Analysis

The values are presented as means±SE. Statistical comparisons of the differences were performed using the one-way or two-way ANOVA for repeated measures combined with Newman-Keuls post hoc test. P<0.05 was considered statistically significant.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results

Ang I and Ang II levels in interstitial fluid, plasma, and kidney are summarized in Figure 1 (n=7). Under resting conditions, basal effluent dialysate concentrations of Ang I and Ang II averaged 0.40±0.02 and 1.56±0.22 nmol/L, respectively. Renal interstitial concentrations of Ang I and Ang II, which were estimated using the in vitro relative equilibrium rates, were 0.84±0.04 and 3.07±0.43 nmol/L, respectively. These values were, in all cases, much higher than respective plasma levels (Ang I, 112±14 pmol/L; Ang II, 106±8 pmol/L; P<0.01, respectively) and kidney contents (Ang I, 178±25 fmoL/g; Ang II, 194±21 fmoL/g; P<0.01, respectively). Furthermore, renal interstitial Ang II concen-
trations were substantially higher than renal interstitial Ang I levels \((P<0.01)\).

The table summarizes the effects of enalaprilat administration on mean arterial pressure (MAP) and plasma Ang I and II levels. Enalaprilat resulted in dose-dependent reductions in MAP. At an infusion rate of 0.2 \(\mu\)mol/kg/min, enalaprilat significantly increased plasma Ang I concentrations. Increasing enalaprilat dosages induced further increases in plasma Ang I concentrations, but there were no significant differences between the enalaprilat-induced increases in plasma Ang I at the 2 higher doses of enalaprilat (1 and 7.5 \(\mu\)mol/kg/min). The lowest dose of enalaprilat (0.2 \(\mu\)mol/kg/min) did not alter plasma Ang II concentrations significantly, whereas, at doses of 1 and 7.5 \(\mu\)mol/kg/min, enalaprilat significantly decreased plasma Ang II concentrations (Table). As shown in Figure 2A, basal plasma ACE activity averaged 65±3 nmol/min/mg/protein and was reduced to almost undetectable levels by enalaprilat infusion (1 and 7.5 \(\mu\)mol/kg/min). In control animals, renal tissue ACE activity averaged 1.7±0.2 nmol/min/mg (n=7). Renal tissue ACE activities in enalaprilat-treated animals (1 and 7.5 \(\mu\)mol/kg/min) were significantly lower than those observed in control animals. The two higher doses of enalaprilat (1 and 7.5 \(\mu\)mol/kg/min) elicited similar decreases in renal tissue ACE activities (Figure 2B). Enalaprilat infusion at a rate of 0.2 \(\mu\)mol/kg/min did not alter interstitial fluid concentrations of Ang I and Ang II significantly. At a rate of 1 \(\mu\)mol/kg/min, enalaprilat significantly increased interstitial fluid concentrations of Ang I from 0.67±0.02 to 0.85±0.03 nmol/L; however, the interstitial fluid concentrations of Ang II were not altered significantly from 3.77±0.25 to 3.65±0.25 nmol/L. Similarly, enalaprilat at a rate of 7.5 \(\mu\)mol/kg/min failed to reduce the interstitial fluid Ang II concentration significantly, although interstitial Ang I levels were significantly increased \((P<0.05\) Figure 3A and 3B).

Acute volume loading with Ringer’s solution for 2 hours significantly lowered hematocrit from 43±3 to 41±2\% (n=5). MAP was not changed during volume expansion...

| Effects of Intra-Arterial Infusion of Enalaprilat on MAP and Plasma Ang I and Ang II Concentrations in Anesthetized Rats |
|--------------------|-----------------|-----------------|-----------------|-----------------|
|                    | MAP (mm Hg)     | Plasma Ang I (pmol/L) | Plasma Ang II (pmol/L) | Ratio Ang II/Ang I |
| Enalaprilat (0.2 \(\mu\)mol/kg/min, n=6) |
| Control            | 118±4           | 122±19           | 138±22           | 1.15±0.23       |
| 120 min            | 104±6*          | 585±94*          | 120±11           | 0.28±0.04*      |
| Enalaprilat (1.0 \(\mu\)mol/kg/min, n=8) |
| Control            | 123±4           | 97±24            | 111±14           | 1.50±0.30       |
| 120 min            | 99±5*           | 1157±216*        | 66±8*            | 0.07±0.02*      |
| Enalaprilat (7.5 \(\mu\)mol/kg/min, n=5) |
| Control            | 114±4           | 133±21           | 110±12           | 0.92±0.16       |
| 120 min            | 68±4*           | 1167±328*        | 67±9*            | 0.09±0.04*      |

Values are expressed as mean±SE. *\(P<0.05\) vs control.

![Figure 1](http://hyper.ahajournals.org/)

Figure 1. Ang I and Ang II levels in renal interstitial fluid, plasma, and kidneys in which microdialysis probes were implanted in anesthetized rats (n=7). Renal interstitial concentrations of Ang I and Ang II were calculated based on equilibrium rates determined in vitro.
As shown in Figure 4A, plasma Ang I and Ang II concentrations were significantly decreased by volume expansion (Ang I, from 110 ± 23 to 36 ± 6 pmol/L; Ang II, from 100 ± 23 to 6 ± 6 pmol/L; \( P < 0.05 \), respectively). In contrast, renal interstitial fluid concentrations of Ang I and Ang II were not altered significantly by acute volume expansion (Figure 4B).

Discussion

The present study demonstrates that renal interstitial fluid Ang II concentrations in anesthetized rats, which were calculated using the relative equilibrium rates determined in vitro, are in the nanomolar range and are much greater than the plasma concentrations. In addition, this study documents that renal interstitial fluid Ang I concentrations are also higher than the corresponding plasma concentrations, although somewhat lower than renal interstitial fluid Ang II concentrations. The finding that renal interstitial fluid concentrations of both Ang I and Ang II are substantially greater than the corresponding plasma concentrations provides further evidence that Ang peptides are locally produced in the kidney.

The present study using the microdialysis technique confirms and extends previous findings showing that Ang II concentrations in renal interstitial fluid in rats are in the nanomolar range. Siragy et al reported that renal cortical interstitial fluid concentrations of Ang II obtained from microdialysis probes are higher, in the range of 20 to 50 nmol/L, suggesting some in vitro conversion of Ang I to Ang II, perhaps because of the long outflow tubes of the microdialysis probes needed for collecting dialysate fluid from the kidneys of conscious animals. To minimize this possibility, we collected fluid directly from the outflow steel tubing of microdialysis probes. Furthermore, the effluent was collected directly into a solution of inhibitors so that dialysate effluent could be immediately mixed with the inhibitors. Finally, dialysate was transferred to glass tubes containing 1 mL chilled 100% methanol immediately after collecting samples. With these procedures, we observed that Ang II concentrations in dialysate samples collected into the inhibitor mixture were significantly higher than the Ang II concentrations in fluid collected without inhibitor. These results indicate that substantive in vitro degradation of Ang II occurred when the lower concentrations of inhibitors were used.

Intraarterial infusion of enalaprilat increased plasma Ang I concentrations by over 10-fold; however, enalaprilat increased renal interstitial concentrations of Ang I by only 20% to 30%. Consistent with previous studies, acute administration of enalaprilat almost completely inhibited plasma ACE activity and significantly decreased plasma Ang II concentrations. However, renal interstitial fluid concentrations of Ang II were unaffected by enalaprilat. These observations are in accordance with those of Dell’Italia and co-workers, who reported that interstitial fluid Ang II in dog heart is much higher than plasma levels. The authors showed that coronary sinus plasma Ang II increased during intravenous Ang I infusion and decreased to baseline after addition of captopril, whereas interstitial Ang II concentrations were not altered during infusion of Ang I or captopril. These observations, as well as the results from the present study, indicate that plasma and interstitial fluid Ang II concentrations are regulated independently, suggesting the compartmentalization of Ang II in the interstitial fluid.

Although the exact mechanisms that explain the high concentrations of Ang I and Ang II in renal interstitial fluid remain unclear, the failure to significantly lower the basal renal interstitial Ang II concentration with enalaprilat suggests that Ang II is produced within the renal interstitium via ACE localized at sites not reached by the administered ACE inhibitor or via non-ACE pathways. Clearly, additional studies are required to determine the source(s) of renal interstitial fluid Ang II.
Ang II, but it should be emphasized that both the present study and previous studies have shown that intrarenal ACE activity is not reduced to the same extent as plasma ACE by infusion of an ACE inhibitor, even at very high doses. This finding suggests that about one half of renal ACE is somehow not accessed by acute ACE inhibition. It is possible that longer-term ACE inhibition progressively reduces intrarenal ACE activity.

To assess the compartmentalization of Ang II in renal interstitial fluid, studies were also performed to determine whether or not interstitial levels of Ang I and Ang II are influenced by changes in plasma concentrations elicited by acute volume expansion. Similar to the results from previous studies, acute volume loading significantly decreased plasma Ang II concentrations by 3-fold. In contrast, acute volume expansion did not significantly reduce renal interstitial concentrations of Ang II. Because acute volume expansion inhibits renin release, we anticipated that renal interstitial concentrations of Ang II would decrease. Although the current methodology might be unable to discern small changes in interstitial Ang II levels, the 3-fold decrease, as occurred in plasma, was not reflected by corresponding changes in interstitial Ang II concentrations. Interestingly, similar results were obtained by Braam et al., who reported that acute volume loading significantly decreased plasma Ang II levels but did not significantly reduce tubular fluid concentrations of Ang II. Although the mechanisms responsible for the maintenance of renal interstitial Ang II levels are not clear, the finding that renal interstitial Ang II levels were not reduced in parallel with plasma Ang II concentrations during acute volume expansion provides further evidence of the compartmentalization and independent regulation of renal interstitial fluid Ang II.

In summary, the present experiments demonstrate that renal interstitial fluid concentrations of Ang I and Ang II are much higher than can be explained on the basis of plasma concentrations. Furthermore, these high interstitial fluid Ang II concentrations are not reduced by acute arterial infusions of ACE inhibitors nor by acute volume expansion, indicating the compartmentalization of renal interstitial fluid Ang II. Such high levels of Ang II in renal interstitial fluid may play an important role in regulating renal function.

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