Renal Interstitial Fluid Angiotensin I and Angiotensin II Concentrations during Local Angiotensin-Converting Enzyme Inhibition

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Abstract. It was recently demonstrated that angiotensin II (AngII) concentrations in the renal interstitial fluid (RIF) of anesthetized rats were in the nanomolar range and were not reduced by intra-arterial infusion of an angiotensin-converting enzyme (ACE) inhibitor (enalaprilat). This study was performed to determine changes in RIF AngI and AngII concentrations during interstitial administration of ACE inhibitors (enalaprilat and perindoprilat). Studies were also performed to determine the effects of enalaprilat on the de novo formation of RIF AngII elicited by interstitial infusion of AngI. Microdialysis probes (cut-off point, 30,000 D) were implanted in the renal cortex of anesthetized rats and were perfused at 2 μl/min. The effluent dialysate concentrations of AngI and AngII were measured by RIA, and reported values were corrected for the equilibrium rates at this perfusion rate. Basal RIF AngI (0.74 ± 0.05 nM) and AngII (3.30 ± 0.17 nM) concentrations were much higher than plasma AngI and AngII concentrations (0.15 ± 0.01 and 0.14 ± 0.01 nM, respectively; n = 27). Interstitial infusion of enalaprilat through the microdialysis probe (1 or 10 mM in the perfusate; n = 5 and 8, respectively) significantly increased RIF AngI concentrations but did not significantly alter AngII concentrations. However, perindoprilat (10 mM in the perfusate, n = 7) significantly decreased RIF AngII concentrations by 22 ± 4% and increased RIF AngI concentrations. Interstitial infusion of AngII (100 nM in the perfusate, n = 7) significantly increased the RIF AngII concentration to 8.26 ± 0.75 nM, whereas plasma AngII and AngII levels were not affected (0.15 ± 0.02 and 0.14 ± 0.02 nM, respectively). Addition of enalaprilat to the perfusate (10 mM) prevented the conversion of exogenously added AngI. These results indicate that addition of AngI in the interstitial compartment leads to low but significant conversion to AngII via ACE activity (blocked by enalaprilat). However, the addition of ACE inhibitors directly into the renal interstitium, via the microdialysis probe, either did not reduce RIF AngII levels or reduced levels by a small fraction of the total basal level, suggesting that much of the RIF AngII is formed at sites not readily accessible to ACE inhibition or is formed via non-ACE-dependent pathways.

There is now substantial evidence indicating that angiotensin II (AngII) is formed locally in the kidney and is critical in the paracrine regulation of renal function and in the pathophysiological processes of hypertension (1–4). Intrarenal AngII formation is particularly complex, because all of the components needed for AngII formation are formed locally but substantial intrarenal metabolism and degradation of AngI and AngII also occur (5). Furthermore, intrarenal AngII is not distributed in a homogeneous manner but is compartmentalized in the tubular fluid (5–8), in interstitial fluid compartments (9–13), and also intracellularly (14–16).

Interstitial AngII plays an important role in the regulation of the renal microcirculation (17–19) and tubular function (19) and acts as a pivotal mediator in the pathogenesis of tubulointerstitial changes (3,4,20–22). Several studies have indicated that AngII is present at high concentrations in renal interstitial fluid (RIF) (9–13). Earlier studies demonstrated that AngII concentrations in the renal lymph were substantially greater than those in arterial or renal venous plasma (9,10). Studies using microdialysis probes implanted in the renal cortex also indicated that RIF AngII concentrations were much greater than plasma concentrations (11–13). A recent study demonstrated that RIF AngI and AngII concentrations in anesthetized rats were in the nanomolar range and were substantially greater than the corresponding plasma concentrations and kidney contents (13). These results support the concept that AngII is formed locally within the renal interstitium or is added to the interstitial compartment. Interestingly, we also observed that the high RIF AngII concentrations were not responsive to acute arterial infusion of an angiotensin-converting enzyme (ACE) inhibitor, enalaprilat. Similarly, acute volume expansion did not lead to significant reductions in RIF AngII concentrations, although plasma AngII levels were significantly decreased (13), suggesting that the regulation of RIF AngII is independent of plasma AngII levels.

This study was conducted to determine whether more direct and local ACE inhibition could modify RIF AngI and AngII concentrations. Experiments were performed in anesthetized...
rats, to determine changes in RIF AngI and AngII concentrations in response to interstitial administration of ACE inhibitors, which were infused through the microdialysis probe by inclusion of the drug in the perfusate. Studies were performed with enalaprilat and a more lipophilic ACE inhibitor, perindoprilat (23,24), to determine effects on RIF AngI and AngII concentrations. Furthermore, we investigated the effects of local administration of enalaprilat on de novo formation of RIF AngII induced by interstitial infusion of AngI substrate.

Materials and 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 260 to 315 g were anesthetized with Inactin (100 to 120 mg/kg, intraperitoneally). The surgical preparation of the animals and basic experimental techniques were identical to those described previously (13,25). During surgical preparation, Ringer’s solution containing 6% bovine serum albumin (BSA) (pH 7.4) was infused intravenously, at a rate of 20 μl/min, to replace volume losses. Ringer’s solution containing 1% BSA (pH 7.4) was then infused, at a rate of 20 μl/min, for the duration of the stabilization and experimental periods.

Characteristics of the Microdialysis Probe

An in vivo microdialysis method (11–13,26) was used for the determination of RIF concentrations of AngI and AngII. The dialysis membrane was made from polyethersulfone fiber (Toyobo Co., Otsu, Japan) measuring 10 mm in length, with a 30,000-D transmembrane diffusion cut-off. Thin steel needles were inserted into both ends of the polyethersulfone fiber. The inflow of each probe was connected to polyethylene tubing (PE-10) and a CMA/100 microinfusion pump (Carnegie Medicine, Stockholm, Sweden). The probes were perfused with Ringer’s solution containing 6% bovine serum albumin (BSA) (pH 7.4) at a rate of 2 μl/min. Before the initiation of each experiment, the probe was perfused for 60 min with Ringer’s solution containing 1% BSA and then soaked in 40% ethanol for 20 min. The dialysate was collected directly from the outflow steel tubing, for analysis of AngI and AngII concentrations. The dead space of the dialysis membrane and outflow steel tubing was <1.0 μl. The tip of the outflow steel tubing was placed into a small tube containing a solution of inhibitors (30 μl of 500 mM ethylenediaminetetraacetate, 15 μl of 1 mM enalaprilat, and 30 μl of 125 mM o-phenanthroline and 0.2 mM pepstatin in 95% ethanol), so that dialysate effluent would be immediately mixed with the inhibitors. We recently demonstrated that use of the inhibitor mixture effectively blocked in vitro generation and degradation of AngII (13). After 30 min of sample collection, the samples were quickly vortex-mixed. Immediately thereafter, 10 μl of mixed solution for AngII measurements and 50 μl of mixed solution for AngI measurements were transferred to two glass tubes containing 1 ml of chilled 100% methanol. These samples were stored at −20°C until the day before analysis.

The efficiency of the microdialysis probe was determined in vitro (11–13,26). At a perfusion rate of 2 μl/min, the relative equilibrium rates for AngI and AngII were 47 ± 1 and 51 ± 1%, respectively, which did not deteriorate with time (13). Because the microdialysis probe was implanted in the renal cortex, it was possible that AngII derived from tubular fluid contributed to the AngII levels in the dialysate. Previously, we administered inulin intravenously, to assess possible contamination of the dialysate by plasma and tubular fluid (13). The dialysate concentration of inulin was below the detectable range (<0.03 mg/ml), indicating that dialysate contamination by tubular fluid and plasma was minimal. Previous studies also demonstrated that dialysate concentrations of AngI and AngII were elevated immediately after implantation of the microdialysis probe but decreased within the first 30 min and then remained stable for up to 270 min (13). Therefore, all in vivo collection experiments were initiated 90 min after implantation of the microdialysis probe.

Effects of Interstitial Infusion of Enalaprilat and Perindoprilat on RIF AngI and AngII Levels

Ninety minutes after implantation of the microdialysis probe, the experimental protocol was initiated with dialysate fluid collections for two consecutive 30-min periods. At the end of the second control sample collection, an arterial blood sample was obtained from a catheter placed in the left femoral artery. Enalaprilat (1 and 10 mM; n = 5 and 8, respectively) or perindoprilat (10 mM, n = 7) was then infused interstitially through the microdialysis probe, by addition of the drug to the perfusate. After 30 min of infusion, four consecutive 30-min dialysate samples were collected. At the end of the final sample collections, arterial blood samples were obtained. The kidney was then removed, and the location of the microdialysis membrane was confirmed by surgical exposure of the probe.

Effects of Interstitial Infusion of Enalaprilat on De Novo Formation of RIF AngII during Interstitial Infusion of AngI

Experiments were performed to determine the effects of local administration of enalaprilat on de novo formation of RIF AngII from AngI infused into the interstitium through the probe (n = 7). During the control period, two interstitial fluid samples and an arterial blood sample were obtained. Then, AngI (100 nM) was infused interstitially through the microdialysis probe. After 15 min of AngI infusion, two consecutive 30-min dialysate samples were collected. A continuous interstitial infusion of enalaprilat (10 mM) was then added to the AngI infusion. After 15 min of enalaprilat infusion, two additional consecutive 30-min sample collections were performed. At the end of each experiment, an arterial blood sample was obtained.

Analytical Procedures

Plasma AngI and AngII levels were measured by RIA, as described previously (7,8,14). The dialysate samples in 100% methanol were evaporated to dryness in a vacuum centrifuge, reconstituted in assay buffer, and assayed by RIA (13).

Statistical Analyses

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

Results

Under resting conditions, the RIF AngI and AngII concentrations calculated by using the in vitro relative equilibrium rates were 0.74 ± 0.05 and 3.30 ± 0.17 nM, respectively (n = 27). These values were, in all cases, much higher than the respective plasma levels (AngI, 146 ± 12 pM; AngII, 138 ± 10 pM; P < 0.01). Furthermore, RIF AngII concentrations were substantially higher than RIF AngI levels (P < 0.01).
Table 1 summarizes the effects of interstitial infusion of enalaprilat and perindoprilat on mean arterial pressure (MAP) and plasma AngI and AngII concentrations. Interstitial infusion of enalaprilat at a dose of 1 mM did not significantly reduce MAP (n = 5); at 10 mM, however, sufficient enalaprilat entered the systemic circulation to significantly decrease MAP (n = 8). Interstitial infusion of perindoprilat (10 mM) also significantly reduced MAP (n = 7). These reductions in arterial pressure indicated that the ACE inhibitors administered via the microdialysis probe infiltrated the renal interstitium and entered the intravascular volume. The average plasma AngI concentrations increased slightly with a dose of 1 mM enalaprilat, but these changes were not statistically significant. Plasma AngII concentrations were not altered by administration of 1 mM enalaprilat. The higher dose of enalaprilat (10 mM) and perindoprilat significantly increased plasma AngI concentrations and decreased plasma AngII concentrations (Table 1). At a dose of 1 mM, enalaprilat infusion for 150 min significantly increased RIF AngI concentrations from 0.67 ± 0.16 nM; however, the RIF AngII concentrations were not significantly altered (from 3.20 ± 0.50 to 3.28 ± 0.50 nM). Similarly, enalaprilat infusion at a dose of 10 mM failed to significantly reduce RIF AngII concentrations, although RIF AngI concentrations were significantly increased from 0.75 ± 0.07 to 1.21 ± 0.11 nM (Figure 1, A and B). As indicated in Figure 1C, enalaprilat significantly decreased the AngII/AngI ratio in the RIF from 4.7 ± 0.8 to 2.8 ± 0.2. In contrast, interstitial infusion of perindoprilat significantly decreased RIF AngII concentrations from 3.36 ± 0.16 to 2.62 ± 0.18 nM, in association with increases in RIF AngI concentrations from 0.77 ± 0.02 to 1.35 ± 0.10 nM (Figure 2, A and B). The AngII/AngI ratio in the RIF was significantly decreased by perindoprilat, from 4.4 ± 0.2 to 2.0 ± 0.1 (Figure 2C).

Interstitial infusion of AngI (100 nM) alone for 75 min did not alter MAP and plasma AngI and AngII levels (n = 7) (Table 1). During AngI infusion, enalaprilat significantly increased plasma AngI concentrations and decreased AngII concentrations (Table 1). AngI infusion for 75 min significantly increased RIF AngII concentrations from 2.85 ± 0.24 to 8.26

Table 1. Effects of interstitial infusion of enalaprilat, perindoprilat, and AngI on mean arterial pressure and plasma AngI and AngII concentrations in anesthetized rats

<table>
<thead>
<tr>
<th></th>
<th>Mean Arterial Pressure (mmHg)</th>
<th>Plasma AngI Level (pM)</th>
<th>Plasma AngII Level (pM)</th>
<th>AngII/AngI Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 5)</td>
<td>116 ± 2</td>
<td>119 ± 18</td>
<td>115 ± 15</td>
<td>1.11 ± 0.12</td>
</tr>
<tr>
<td>Enalaprilat (1 mM in perfusate, 150 min)</td>
<td>114 ± 3</td>
<td>153 ± 13</td>
<td>100 ± 23</td>
<td>0.67 ± 0.15</td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>115 ± 4</td>
<td>202 ± 24</td>
<td>172 ± 20</td>
<td>0.86 ± 0.06</td>
</tr>
<tr>
<td>Enalaprilat (10 mM in perfusate, 150 min)</td>
<td>106 ± 4b</td>
<td>1246 ± 251b</td>
<td>122 ± 20b</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>108 ± 5</td>
<td>95 ± 7</td>
<td>106 ± 18</td>
<td>1.11 ± 0.15</td>
</tr>
<tr>
<td>Perindoprilat (10 mM in perfusate, 150 min)</td>
<td>99 ± 5b</td>
<td>690 ± 143b</td>
<td>74 ± 11b</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>110 ± 4</td>
<td>147 ± 24</td>
<td>138 ± 21</td>
<td>1.12 ± 0.25</td>
</tr>
<tr>
<td>AngI (100 nM in perfusate, 75 min)</td>
<td>110 ± 4</td>
<td>152 ± 19</td>
<td>134 ± 15</td>
<td>0.99 ± 0.17</td>
</tr>
<tr>
<td>AngI plus enalaprilat (10 mM in perfusate, 75 min)</td>
<td>100 ± 3b</td>
<td>1603 ± 246b</td>
<td>94 ± 12b</td>
<td>0.06 ± 0.01</td>
</tr>
</tbody>
</table>

*Values are means ± SEM. AngI, angiotensin I.

b P < 0.05 versus control.
0.75 nM, demonstrating significant conversion of the infused AngI (Figure 3). The addition of enalaprilat to the AngI infusion prevented the increases in RIF AngII concentrations caused by the administration of exogenous AngI but did not decrease RIF AngII levels below the basal values (3.41 ± 0.31 nM).

**Discussion**

Several studies have indicated that RIF AngII exerts biologic effects, including the regulation of renal function (2,17–19), and may cause tubulointerstitial injury when levels are inappropriately elevated (3,4,20–22). Mitchell and Navar (18,19) demonstrated that AngI and AngII added to the renal interstitium via perfusion into peritubular capillaries caused afferent arteriolar vasoconstriction, decreases in the single-nephron GFR, and increases in the proximal fractional reabsorption rate. These results indicate the occurrence of significant diffusion of AngII from the peritubular capillaries and conversion of exogenously added AngI to AngII in the renal interstitium. Consistent with our recent data (13), the studies presented here confirm that RIF AngII concentrations are in the nanomolar range and are substantially greater than the corresponding plasma concentrations. In addition, it seems clear that, when exogenous AngI is delivered via the microdialysis probe, interstitial ACE converts some of it to AngII. However, under basal conditions, the addition of ACE inhibitors directly into the renal interstitium via the microdialysis probe either did not reduce the RIF AngII concentration or reduced it by only a small fraction of the total basal level. These results support the previously suggested concept (13) that much of the RIF AngII is formed at sites that are not readily accessible to ACE inhibition or is formed via non-ACE-dependent pathways. These results in the kidney are consistent with those noted in the heart by Dell’Italia et al. (26), who demonstrated that interstitial fluid concentrations of AngII in the heart were not reduced by infusion of the ACE inhibitor captopril. That study also demonstrated that captopril almost completely inhibited the interstitial ACE activity (26).

The source of the AngII collected from the renal interstitium remains unclear. Previous studies demonstrated that when 14C-labeled AngII was administered into the renal artery, no significant amount of 14C-labeled AngII appeared in the renal lymph, suggesting that most of the AngII in renal lymph is not derived from circulating AngII (9). Therefore, it seems that most of the RIF AngII is formed from locally generated AngI. Collectively, these observations suggest that, under basal conditions, high concentrations of interstitial AngII are formed via ACE localized at sites that are not accessible to the administered ACE inhibitors or via non-ACE pathways.

In this study, we perfused enalaprilat and perindoprilat in-
terstitially, through the microdialysis probe, by adding the drugs to the perfusate containing 10% BSA. It is thus possible that differences in the protein binding ratios of these drugs might account for the observation that RIF AngII concentrations were significantly decreased by perindoprilat but not by enalaprilat. The protein binding of enalaprilat is much greater than that of perindoprilat; therefore, a greater amount of perindoprilat might have traversed the renal interstitium. However, we infused enalaprilat and perindoprilat at very high concentrations (10 mM), and the MAP was significantly decreased during interstitial infusion of both enalaprilat and perindoprilat. These data suggest that both ACE inhibitors infiltrated and entered the intravascular volume. It is thus reasonable to conclude that this concentration is sufficient to inhibit the activity of ACE existing in the RIF around the microdialysis probe. Nevertheless, the differences in the effects of enalaprilat and perindoprilat might be the result of differences in their binding to BSA, in their affinity for interstitial ACE, or in their ability to diffuse within the interstitium.

Recent studies suggested that significant amounts of renal AngII are formed intracellularly (5,14–16), and it is possible that the intracellularly produced AngII is secreted into the interstitial space. In this study, we also investigated changes in RIF AngII concentrations in response to local administration of the lipophilic ACE inhibitor perindoprilat (23,24). It is possible that some of the administered perindoprilat is distributed more uniformly within the interstitium and partially permeates cell membranes. We observed that locally administered perindoprilat significantly decreased RIF AngII concentrations by 0.75 ± 0.22 nM, but the decrease was still only a small fraction of the basal value (3.36 ± 0.16 nM). To the extent that ACE bound to the tubular basolateral membranes is responsible for the formation of AngII in the renal interstitium (2,5), it is possible that the convective force attributable to continuous tubular fluid reabsorption might limit the concentrations of ACE inhibitors that can be achieved at or near the basolateral membranes.

In this study, we observed that interstitial infusion of AngI significantly increased RIF AngII concentrations. Furthermore, these increases were prevented by coadministration of enalaprilat. These data indicate that interstitial ACE converts exogenously administered AngI. As already stated, however, the increases in RIF AngI levels during enalaprilat infusion were not accompanied by changes in RIF AngII concentrations. At present, we do not have a satisfactory explanation for why enalaprilat resulted in increases in RIF AngI levels without reductions in RIF AngII levels. However, it seems important to recognize that basal RIF concentrations of AngI were consistently lower than those of AngII and that the increases in AngI levels after ACE inhibition were quantitatively modest, compared with RIF AngII concentrations. Therefore, it is possible that blockade of the conversion of AngI to AngII in the interstitial compartment might not be reflected by significant reductions in RIF concentrations of AngII. It should also be noted that ACE inhibitors have been demonstrated to increase both AngI and Ang-(1–7) levels in vitro (27) and in vivo (28). AngI can be converted directly to Ang-(1–7) (29–31), which circulates in the blood at concentrations similar to those of AngII (30,31). Substantial increases in interstitial fluid Ang-(1–7) levels during interstitial infusion of AngI in dog heart were recently reported (32). Furthermore, Wei et al. (33) reported that administration of the ACE inhibitor ramipril did not alter interstitial AngII concentrations but significantly increased interstitial Ang-(1–7) concentrations in rat heart. Although we did not measure Ang-(1–7) levels in this study, those data suggest the possibility that, during local ACE inhibition, some of the increased interstitial fluid AngI is converted to Ang-(1–7), rather than AngII. Nevertheless, shunting to this possible pathway does not explain why RIF AngII levels failed to decrease.

Although AngII formation in rodents is primarily ACE-related (2), non-ACE-dependent AngII formation has also been observed in rat heart (34) and vasculature (35,36). Guo et al. (36) demonstrated that chymase expression and chymostatin-inhibitable ACE activity were significantly increased in spontaneously hypertensive rats, suggesting a possible role for this enzyme in AngII formation in rats. ACE-related carboxypeptidase (ACE2) is also expressed in rat kidney (37). Interestingly, recent studies demonstrated that ACE2 generated Ang-(1–9) from AngI but did not further convert Ang-(1–9) to AngII (38). However, the extent to which these processes regulate RIF AngI and AngII remains unknown.

In conclusion, this investigation demonstrated the de novo formation of RIF AngII with local AngI administration, via ACE activity (blocked by enalaprilat). Nevertheless, the finding that local ACE inhibition either did not reduce RIF AngII levels or reduced levels by a small fraction of the total basal levels suggests intrarenal formation of AngII via ACE localized at sites not easily accessed by the administered ACE inhibitors and/or via non-ACE pathways.

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References


