Effect of Captopril on the Release of the Components of the Renin-Angiotensin System into Plasma and Lymph

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ABSTRACT

The effects of captopril on the intrarenal renin-angiotensin system were assessed from measurements in arterial plasma, renal venous plasma, and renal lymph from salt-depleted dogs. In the basal state, immunoreactive angiotensin II (Ang II) in renal venous plasma averaged only 60 ± 12% (P < 0.01) of arterial plasma, although the concentration of Ang II in renal lymph was 2.0 ± 0.4-fold (P < 0.05) greater. The Ang II concentration of renal lymph incubated ex vivo at 37°C doubled in 10 to 15 min, which was the time taken to collect renal lymph samples. Compared with arterial plasma, renal lymph contained lower concentrations (P < 0.01) of renin substrate and angiotensin-converting enzyme but higher concentrations of active (5.3 ± 2.1-fold) and inactive (8.9 ± 3.2-fold) renin. Although captopril increased the secretion of active renin into renal venous plasma by six-fold, the secretion of total renin was unchanged because of a reciprocal fall in the secretion of inactive renin. The percent reduction in renal vascular resistance with captopril correlated with the percent fall in Ang II in renal lymph (γ = 0.70). In conclusion: (1) all components of the renin-angiotensin system are represented in the renal interstitium, as reflected in lymph; (2) Ang II concentrations in renal lymph in vivo approximate arterial levels; (3) increased secretion of active renin into plasma during intrarenal infusion of captopril into denervated kidneys is due predominantly to renin activation; and (4) renal vascular resistance may depend on the concentration of Ang II in the renal interstitium.

Key Words: Captopril, RBF, renin—active and inactive, angiotensin, renin substrate, converting enzyme activity, lymph—renal

Angiotensin II (Ang II) can exert important local intrarenal actions. Thus, intrarenal infusions of Ang II antagonists in salt-depleted animals evoke ipsilateral renal vasodilation, increase in GFR, natriuresis, and renin release (1), whereas Ang II itself, whether infused i.v. or applied extraluminally, causes vasoconstriction of the preglomerular and postglomerular resistance vessels of intact or hydronephrotic rat kidneys (2,3). However, because of the rapidity of RBF, only some 20 to 25% of Ang I in arterial plasma is converted to Ang II during passage through the kidney (4). Much locally formed renin may be secreted into the renal interstitium before escaping into peritubular capillaries or lymphatics (5). Therefore, the renal interstitial renin-angiotensin system (RAS) could contribute to the regulation of renal function. The renal lymphatics are distributed preferentially to the resistance vessels, glomeruli, and juxtagonalular apparatus of the renal cortex in the dog (6). Although previous studies have shown that renin or Ang II levels are higher in renal lymph than in renal venous blood (7-12), a full characterization of the RAS in lymph from the dog kidney has not been reported. Therefore, the first aim of these studies was to contrast measurements in arterial and renal venous plasma and renal lymph of active and inactive renin, renin substrate (RS), angiotensin-converting enzyme (ACE) activity, and Ang II. We used a preparation that allows collection of the entire output of lymph from a denervated kidney to permit comparison of the net rates of release of the components of the RAS into plasma and lymph (12).

Changes in PRA after captopril have been proposed as a provocative test for renovascular hypertension (13,14). Although captopril can increase peripheral levels of active renin, the levels of inactive renin are variously reported to remain unchanged (15,16) or to fall (17-20). Reciprocal changes in active and inactive renin concentrations could imply that captopril administration altered renal renin activation, rather than net renin release. We have characterized an active and inactive renin system in dog plasma and
renal lymph (9). Therefore, the second aim of these studies was to investigate the effects of short-term administration of captopril on the secretion of active and inactive renin into renal plasma of the dog.

METHODS

Preparation of the Animals

Experiments were performed on adult greyhounds weighing 30.7 ± 0.7 kg (mean ± SE). For 5 days, dogs were fed a standard diet of biscuits and water. At 42 and 18 h before experiments, they were given 40 mg of furosemide with their food; after the second dose, food and water were withheld to ensure that the animals became fluid depleted and salt depleted. We have found that this protocol stimulates the RAS even within a denervated, autotransplanted kidney preparation as used in this study (21).

On the day of the experiment, dogs were anesthetized with pentobarbitone sodium. The trachea was intubated, and the arterial blood Pco₂ was maintained at circa 32 mm Hg by adjustment of a mechanical ventilator (Harvard animal ventilator; Harvard Apparatus, South Natick, MA). Mean arterial blood pressure (MAP) was recorded by a femoral arterial cannula connected to a pressure transducer (Statham Instruments, Oxnard, CA). The experimental kidney was autotransplanted to the neck to ensure denervation and to allow collection of all of the renal lymph (12,21). Renal ischemia lasted 4 to 6 min. The kidney was housed in a watertight plexiglass box filled with light mineral oil. Lymph issuing from the incised lymphatic ducts was aspirated every 10 min from the bottom of the box by a cannula. Renal venous blood was sampled from a cannula passed retrograde into a tributary of the jugular vein draining from the transplanted kidney.

A solution of 0.15 M NaCl solution was infused via a needle in the artery supplying the kidney at 0.1 mL·min⁻¹·kg⁻¹ body wt. The pressure recorded from the needle (renal perfusion pressure) was found to equal the arterial pressure in each dog. As in previous studies, the animals received 15 mL·kg⁻¹ body wt of 0.15 M NaCl to replace perioperative fluid losses, and thereafter, they received an I.V. infusion of 0.36 M mannitol in 0.15 M NaCl delivered at 0.04 mL·min⁻¹·kg⁻¹ body wt. This protocol has been used previously to maintain the urine flow rate (UV) at 0.5 to 1.0 mL·min⁻¹ to facilitate clearance measurements (1,9,12,21). Inulin and para-aminohippuric acid (PAH) were infused to estimate GFR and RPF, respectively. Inulin was administered as a bolus dose of 500 mg followed by a sustaining infusion of 70 mg·h⁻¹. PAH was administered as a bolus dose of 250 mg followed by a sustaining infusion of 100 mg·h⁻¹. Values of RPF were corrected for the measured renal extraction of PAH by the use of samples of renal venous plasma. One hour elapsed after completion of surgery before any measurements were undertaken. In previous studies with this same preparation, we found that the RBF, electrolyte excretion, UV, and urine osmolarity of the transplanted kidney did not differ from that in the in situ kidney and that these parameters as well as the rates of secretion of active and inactive renin into plasma and lymph and the arterial, renal venous, and renal lymph concentrations of Ang II remained stable over a 2-h period (1,9,12,21). Therefore, additional time controls were not undertaken.

Protocols

Series I: Effects of Captopril on Ang I-Induced Renal Vasconstriction. The ability of captopril to inhibit ACE activity in vivo was studied in five dogs. Peak changes in RBF were recorded from a noncanulating arterial blood flow probe (Biotronix, Inc., Hollywood, FL), placed 2 inches proximal to the intrarenal arterial needle during intrarenal-arterial injections of 0.2 mL of solutions of Ang I (0.02, 0.2, 1, 5, 25, and 100 µg) in 0.15 M NaCl. Doses were given in random order at 5-min intervals, except that the highest dose (which reduced RBF by more than 50%) was given at the end of each experiment to prevent carryover effects. After a dose-response relationship was established, captopril was given intrarenally as a bolus of 1.5 mg·kg⁻¹ body wt followed by a constant infusion of 0.7 mg·kg⁻¹ body wt·h⁻¹. After 45 min, the Ang I dose-response study was repeated. Log-dose response curves were constructed from which the ED₅₀ for RBF was read.

Series II: Measurement of Renal Function and the Components of the RAS and the Effect of Captopril on These. The aims of these studies were: (1) to measure the concentrations and rates of release into renal venous plasma and renal lymph before and after administration of captopril of total renin, active renin, inactive renin, RS, ACE activity and Ang II; and (2) to relate changes in renal vascular resistance (RVR) induced by captopril to changes in the concentration of Ang II in blood or lymph.

Seven dogs were studied. They were separate from those of group 1. Basal measurements were made during an intrarenal-arterial infusion of 0.15 M NaCl at 0.1 mL·min⁻¹·kg⁻¹ body wt. Total collections of urine and renal lymph lasted 30 to 45 min, depending on the lymph flow rate (LFR). Femoral arterial and renal venous blood sample were obtained at the midpoint. Thereafter, captopril (dissolved in 0.15 M NaCl) was administered into the renal artery in a loading dose of 1.5 mg·kg⁻¹ body wt followed by a constant intrarenal infusion of 0.7 mg·kg⁻¹ body wt·h⁻¹. After 45 min equilibration, the measurements
were repeated during a 30- to 45-min experimental period.

Processing of Plasma and Lymph

Renal lymph was aspirated every 10 min. Samples were kept on ice, and those obtained during each clearance period were pooled. Blood was sampled into chilled syringes. Blood and lymph were decanted into chilled tubes containing EDTA and were centrifuged at 4°C. One-milliliter samples for Ang II assay were decanted into DFP-containing tubes (22). Blood and lymph samples for estimation of ACE activity were placed in plain tubes and left to stand on ice for 2 h. Therefore, they were centrifuged and the serum and lymph were separated. All samples were stored at −70°C.

Determinations of the Concentrations of Total, Active, and Inactive Renin, RS, ACE Activity, and Ang I and Ang II Concentrations

For determination of the active plasma renin concentrations and active lymph renin concentrations, the rates of generation of Ang I from 100-μL samples were estimated in the presence of an excess (250 μL) of exogenous dog’s substrate. Substrate was obtained as a twofold dilution of plasma from a nephrectomized dog (9). The Ang I-generating reaction was performed at pH 7.4 for 3 h at 37°C, as described in detail (9). Results are expressed as nanograms Ang I generated per milliliter per hour.

For determination of total (active plus inactive) renin concentrations in plasma and renal lymph, 100 μL of sample was incubated with 10 μL of trypsin (final concentration of 0.6 −2 mg·ml⁻¹) for 1 h at 4°C (23). Trypsin (T-8253; Sigma Chemical Co., St. Louis, MO) was dissolved in 0.001 N HCl and 5% BSA. After 1 h, the reaction was stopped by the addition of 10 μL of soybean trypsin inhibitor (Sigma; 5 mg·ml⁻¹) and 5% phenylmethlysulfonyl fluoride (5 μL) for 15 min at 25°C. These methods have been validated in our laboratory with samples of dog plasma and renal lymph by use of renin-specific monoclonal antibodies, immune affinity, and affigel blue chromatography (9). The increased rate of generation of Ang I after trypsin treatment of plasma and renal lymph was shown to result from activation of inactive renin and could not be attributed to cleavage of angiotensin from substrate by trypsin (9). The inactive renin concentration was obtained by subtraction of the active fraction from the total.

For determination of RS concentration, 100 μL of sample was incubated at room temperature in the presence of inhibitors of angiotensinase and with addition of excess renin until no further Ang I generation occurred; preliminary studies showed that 1 h was sufficient. Substrate concentration was expressed as nanograms Ang I generated per milliliter of sample per hour of incubation.

For determination of ACE activity in vitro, 50 μL of serum or lymph was assayed with [³H]hippuryl-glycine as the substrate (24).

For determination of Ang II, 1-mL samples of plasma and lymph were decanted into DFP-containing tubes and were frozen at −70°C for subsequent extraction and RIA with highly selective antiserum (22). The cross-reactivity of the breakdown peptides of Ang II (1-4, 1-6, and 1-7) are 0.6, 1.6, and 0.4%, respectively. Nussberger et al. (25) have shown that prior extraction of Ang II from human plasma by HPLC reduced the blank value in the subsequent RIA from peptides that reacted with their Ang II antibody. They found that the short-term administration of an ACE inhibitor reduced the plasma Ang II concentration by 5.2 fmol·mL⁻¹ when assayed after HPLC extraction and by 4.2 fmol·mL⁻¹ when assayed without HPLC extraction. This suggests that our method may overestimate basal levels of Ang II but should be quite reliable in detecting short-term changes induced by ACE inhibition.

For determination of the rate of Ang II generation ex vivo, two 5-mL samples of renal lymph obtained from separate dogs and collected in the basal state were dialyzed for 12 h at 0°C against 0.1 M Tris buffer at pH 7.4 to remove preformed peptides. Thereafter, samples were diluted 1:1 with 0.1 M Tris buffer containing sodium tetraionate and phenylmethylsulfonyl fluoride and incubated at 37°C for 0, 1, 3, 10, 100, and 300 min when samples were boiled and centrifuged and the supernatant was assayed for Ang II concentration.

Calculations

GFR was calculated from the clearance of inulin and RPF from the clearance of PAH corrected for renal extraction. RVR was calculated from MAP factored by RBF. The rate of renin secretion into plasma was calculated from the renal venous minus arterial concentrations and multiplied by the RPF, whereas the rate of renin secretion into lymph was calculated from the renal lymph minus arterial concentrations multiplied by the LFR.

Statistical Methods

Results are presented as mean ± SE. Within-dog comparisons were assessed with a paired t test and between-group comparisons with an unpaired t test. The Bonferroni adjustment for repeated analyses was applied (26), and values were considered significant at P < 0.025.
RESULTS

Series 1: Effects of Captopril on Renal Vasocostriction with Ang I

The effects of captopril on ACE activity in vivo were assessed from changes in Ang I-induced renal vasocostriction. Ang I did not change the MAP except for an inconsistent increase at the highest dosage. Ang I led to a dose-dependent fall in RBF (Figure 1). After captopril, the dose-response curve was shifted to the right without an apparent change in slope; the ED50 for Ang I increased from 1.2 to 10 μg. In preliminary studies, we found that a lower dose of captopril (0.5 mg·kg⁻¹ body wt intrarenally) did not produce such consistent inhibition of Ang I-induced renal vasocostriction, and therefore, the larger dose was selected for this study. Higher doses of captopril would clearly be required to produce full inhibition of ACE activity in the kidney, but this was not investigated.

Effects of Captopril on Renal Function

Basal renal function (Table 1) was comparable to several previous series using this model (1,9,12,21). After captopril, there was a consistent increase in RBF despite a reduction in MAP; consequently, RVR fell substantially. The GFR was maintained, and therefore, the filtration fraction fell sharply. Captopril did not change UV or sodium excretion. Basal renal function (Table 1) was comparable to several previous series using this model (1,9,12,21).

The effects of captopril on MAP, renal function, and renal lymph flow are shown in Table 1. Mean ± SE values (N = 7) and changes produced by captopril for MAP, GFR, RPF, filtration fraction (FF), RBF, RVR, UV, sodium excretion (UnOv), and renal LFR. NS, not significant.

Table 1. Effects of captopril on MAP, renal function, and renal lymph flow

<table>
<thead>
<tr>
<th>Initial Value</th>
<th>Change During Captopril</th>
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<tr>
<td>MAP (mm Hg)</td>
<td>164 ± 6 P &lt; 0.01</td>
</tr>
<tr>
<td>GFR (ml·min⁻¹·kg⁻¹ body wt)</td>
<td>1.19 ± 0.09 -0.18 ± 0.09 NS</td>
</tr>
<tr>
<td>RPF (ml·min⁻¹·kg⁻¹ body wt)</td>
<td>3.94 ± 0.38 +1.07 ± 0.25 P &lt; 0.01</td>
</tr>
<tr>
<td>FF (%)</td>
<td>32.7 ± 3.5 P &lt; 0.001</td>
</tr>
<tr>
<td>RBF (ml·min⁻¹·kg⁻¹ body wt)</td>
<td>8.61 ± 0.83 +2.40 ± 0.51 P &lt; 0.01</td>
</tr>
<tr>
<td>RVR (mm Hg·ml⁻¹·min⁻¹·kg⁻¹ body wt)</td>
<td>21.8 ± 2.6 -6.7 ± 0.9 P &lt; 0.001</td>
</tr>
<tr>
<td>UV (μl·min⁻¹·kg⁻¹ body wt)</td>
<td>37.2 ± 9.2 -10.7 ± 6.9 NS</td>
</tr>
<tr>
<td>UnOv (μmol·min⁻¹·kg⁻¹ body wt)</td>
<td>1.60 ± 0.63 -0.26 ± 0.73 NS</td>
</tr>
<tr>
<td>LFR (μl·min⁻¹·kg⁻¹ body wt)</td>
<td>4.79 ± 0.55 -0.02 ± 0.89 NS</td>
</tr>
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</table>

Figure 1. Mean (± SE) values from five dogs are shown for changes in RBF (assessed by electromagnetic flowmeter) of the experimental kidney as a function of the intrarenal dose of Ang I delivered into the renal artery. Values for control dogs (solid circles and solid lines) are contrasted with those for dogs receiving captopril (1.5 mg·kg⁻¹ body wt followed by intrarenal infusion of 0.7 mg·kg⁻¹ body wt·h⁻¹; open symbols and broken lines).

Renin Concentration and Release

Data for renin concentrations are shown in Table 2. In the basal state, the total renin concentration of venous plasma was significantly (P < 0.02) higher than that of arterial plasma (average, 21%); the active renin concentration was similar in arterial and venous plasma. The total renin concentration of venous lymph was consistently above that in arterial plasma; the mean increase was 3.44 ± 0.88-fold (P < 0.01). This was because of higher levels of both active renin concentration (5.3 ± 2.1-fold) and inactive renin concentration (8.9 ± 3.2-fold). The fraction of the total renin concentration that was present in active form was similar in arterial and venous plasma and in renal lymph.

The effect of captopril on arterial plasma was to increase the active renin by 68% without causing consistent changes in the inactive renin (Table 2). The effects of captopril on the venous plasma were more marked; the active renin concentration of venous plasma more than doubled, whereas the inactive renin concentration tended to fall. Captopril increased the fraction of renin in active form in venous plasma from 37 to 68%.

Captopril did not produce a consistent change in...
the concentration of renin in renal lymph (Table 2). Before captopril, the active renin concentration in lymph was 5.33 ± 2.1-fold higher than in arterial plasma. During captopril, this ratio was reduced to 2.1 ± 0.4-fold.

As shown in Figure 2 and reported previously (1,9,12,21), the rates of secretion of active renin into renal venous plasma with this denervated dog's kidney model were low. The active and inactive renin concentrations in renal lymph were invariably higher than in arterial plasma, but because of the much lower rate of lymph flow compared with plasma, the net rates of secretion of renin into lymph were quite low. As shown in Figure 2, captopril increased the active renin secretion rate into renal plasma by six-fold; this was due to a combination of a doubling of the active renin concentration in renal venous plasma and an increase in RPF. In contrast, captopril did not change the total renin secretion rate and reduced the inactive renin secretion rate (70 ± 18 to 18 ± 10 ng of Ang I·mL⁻¹·h⁻¹/mL·min⁻¹·kg⁻¹, P < 0.02). Therefore, there were reciprocal changes in the rates of secretion of active and inactive renin into plasma after captopril administration. Captopril did not cause significant changes in the rates of secretion of total, active, or inactive renin into renal lymph.

**TABLE 2. Effects of captopril on the concentrations of total, active, and inactive renin**

<table>
<thead>
<tr>
<th></th>
<th>Arterial Plasma</th>
<th>Renal Venous Plasma</th>
<th>Renal Lymph</th>
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<tbody>
<tr>
<td>Total Renin (ng·h⁻¹·mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>18.7 ± 8.9</td>
<td>23.8 ± 10.6</td>
<td>94.1 ± 46.7</td>
</tr>
<tr>
<td>During</td>
<td>30.3 ± 12.4</td>
<td>36.0 ± 13.8</td>
<td>71.0 ± 31.1</td>
</tr>
<tr>
<td>Change</td>
<td>+11.6 ± 3.8</td>
<td>+12.2 ± 4.6</td>
<td>-23.0 ± 20.0</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.025</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Active Renin (ng·h⁻¹·mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>12.4 ± 7.3</td>
<td>12.4 ± 6.8</td>
<td>45.7 ± 22.6</td>
</tr>
<tr>
<td>During</td>
<td>20.7 ± 8.9</td>
<td>26.7 ± 9.8</td>
<td>47.8 ± 24.2</td>
</tr>
<tr>
<td>Change</td>
<td>+8.4 ± 3.4</td>
<td>+14.3 ± 4.6</td>
<td>+2.2 ± 22.5</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Inactive Renin (ng·h⁻¹·mL⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>6.3 ± 2.0</td>
<td>11.5 ± 4.9</td>
<td>48.4 ± 24.2</td>
</tr>
<tr>
<td>During</td>
<td>9.5 ± 4.4</td>
<td>9.3 ± 3.7</td>
<td>23.2 ± 9.7</td>
</tr>
<tr>
<td>Change</td>
<td>+3.2 ± 2.6</td>
<td>-2.1 ± 3.0</td>
<td>-22.7 ± 19.6</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Mean ± SE values (N = 7). Before, during basal state; During, during intrarenal captopril.

**Figure 2. Mean (± SE) values from seven dogs are shown for secretion rates of active and total renin into plasma or renal lymph measured in the basal state (open boxes) or after administration of captopril (hatched boxes). Al, Ang I.**
ACE Activity, RS, and Ang II

The effects of captopril on ACE activity, RS, and Ang II concentrations are shown in Table 3. Considering first the values measured in the basal state, both ACE activity and RS were significantly \( P < 0.01 \) lower in renal lymph than in arterial plasma, averaging only 51 ± 12 and 55 ± 7%, respectively. Despite this, the basal Ang II concentrations were 2.0 ± 0.4-fold higher \( P < 0.01 \) in lymph than in arterial plasma. Comparing values in renal venous and arterial plasma, there were no differences for ACE activity or RS but Ang II was always lower in renal venous plasma, averaging 60 ± 12% \( P < 0.02 \).

The intrarenal administration of captopril did not change the ACE activity of arterial plasma, but it reduced the ACE activity of renal venous plasma and renal lymph by 30 and 59%, respectively. Unlike the effects of prolonged captopril administration (27), captopril increased RS modestly in arterial and renal venous plasma. Captopril invariably reduced Ang II concentrations in arterial and renal venous plasma and in renal lymph.

Samples of renal lymph were collected over a 10-min period. Because all of the components of the RAS were detected in lymph, this could allow generation of Ang II \textit{ex vivo}. Therefore, two samples of lymph were dialyzed against buffer at 0°C overnight to remove preformed Ang and were incubated for up to 300 min at 37°C. As shown in Figure 3, generation of Ang II increased with time up to 100 min. After 10 min of incubation, the Ang II concentrations in each sample had increased by about 90%.

There were no significant correlations between absolute changes in RVR and Ang II concentrations in arterial or renal venous plasma or lymph. However, basal values of RVR and Ang II concentration varied considerably between dogs, and when analyzed as fractional changes, there was a significant correlation between captopril-induced reductions in RVR and Ang II concentrations in renal lymph (Figure 4). In contrast, no significant correlations were apparent in the small number of dogs studied between frac-
Figure 4. Individual values for fractional changes in RVR are shown as a function of fractional changes in the concentration of Ang II in renal lymph during administration of captopril.

Discussion

The main findings of this study are as follows. Renal lymph contains a complete RAS and can generate Ang II rapidly. The higher concentrations of Ang II in renal lymph compared with that in arterial plasma in this preparation can be ascribed to ex vivo generation during sample collection. Compared with plasma, lymph contains higher levels of active and inactive renin but lower levels of ACE activity and RS. Captopril infusion causes reciprocal increases in the secretion of active and inactive renin into plasma without changing the total renin secretion rate. After captopril, there is a correlation between fractional changes in Ang II concentration in renal lymph and RVR.

As in previous studies (4,12,28–30), the renal venous plasma Ang II concentration was well below that in arterial plasma. In view of the high rates of blood flow to the kidney, this large negative venous–arterial plasma gradient for Ang II implies that the kidney is of major importance for the clearance of arterial Ang II. Studies with isotopically labeled Ang indicate that 70 to 90% of Ang I or II delivered to the kidneys is degraded in a single passage (4,28,30). This rate of degradation is so high that the majority of the Ang II present in renal venous blood is formed de novo within the kidney despite the reduced Ang II concentrations found in the renal vein (30). Levels of Ang II in renal lymph were also higher than in arterial or renal venous plasma, confirming previous reports (7,8,12). The higher levels of Ang II and renin in renal lymph cannot be attributed to their filtration from plasma because renin or radiolabeled Ang II infused into the systemic circulation does not appear in renal lymph (8,11). The Ang II in lymph probably reflects local synthesis because our results show that lymph contains all of the components required for generation of Ang II and has a significant capacity for Ang II generation ex vivo. Indeed, this capacity for Ang II generation in lymph can lead to an overestimation of the Ang II concentrations in the renal interstitium, as reflected in renal lymph. Thus, the renal lymph Ang II concentrations reported in some earlier studies in the dog were 5- to 10-fold higher than those reported here; these protocols entailed 30- to 60-min collection periods during which Ang II generation may have been occurring ex vivo in the sample container (8). In this study, we could account for all of the excess concentration of Ang II in lymph above that in plasma by ex vivo generation during lymph sampling, with the data from two sets of lymph collected in the basal state. It is possible that some loss of renin activity or RS may have occurred during the dialysis of the two renal lymph samples in our studies, which may have underestimated the degree of ex vivo Ang II generation in renal lymph. Ang II generation in the kidney in vivo is a dynamic process that may proceed at different rates in various compartments. Indeed, a recent study in the rat has reported that Ang II concentrations in efferent arteriolar plasma and glomerular filtrate may be up to 1,000-fold higher than that in arterial plasma (31). These conclusions depend critically on the assumption of no ex vivo generation of Ang II in the microsamples.
Although the study presented here has not confirmed any such dramatic increases in Ang II concentrations within the kidney, different compartments were sampled in the two studies. Another study in the rat reported very high values for Ang I and renin activity in renal lymph. However, lymph samples were collected over 2 to 4 h and values were related to thoracic lymph obtained from nephrectomized rats. Thus, the relationships between plasma and renal lymph concentrations of Ang I and renin cannot be deduced clearly from that previous study (10).

Previous studies in human subjects given captopril have shown that plasma levels of active renin increase, but the effects on inactive renin have been inconsistent (15–20). Thus, Derkx et al. (17) report a striking rise in active renin and a fall in inactive renin after captopril. These results were confirmed with measurements of renin activation with cold (18), trypsin (19,20), or acid (20). In contrast, neither Sealey et al. (16) nor Millar et al. (15) found a reduction in the plasma inactive renin concentration after captopril administration to hypertensive subjects. This controversy has been ascribed to different time courses of sampling between studies (20). Moreover, high doses of ACE inhibitors are needed to fully block intrarenal generation of Ang II in the dog, as shown by Rostvall et al. (30). In the study presented here, although captopril was given intrarenally, it blunted, but certainly did not prevent, Ang I-induced renal vasoconstriction (Figure 1) and therefore presumably was insufficient to provide complete inhibition of intrarenal ACE activity. This was confirmed by the blunted, but not abrogated, levels of ACE activity measured in renal lymph and renal venous plasma during the intrarenal captopril infusion. The previous studies measured only plasma concentrations of renin. Therefore, to approach the problem more directly, we measured the rates of renal secretion of active and inactive renin after captopril. Our results did indeed show that the measurement of peripheral plasma renin shortly after administration of captopril may mask important changes in the pattern of renin secretion by the kidney. Thus, 45 to 90 min after administration of an intrarenal dose of captopril, there was an increase in the systemic concentration of active renin without significant changes in inactive renin, whereas with transrenal sampling, we found that captopril had induced a large increase in the rate of secretion of active renin and a matching fall in the rate of secretion of inactive renin (Figure 2). Presumably, these changes in renin secretion require some time to become apparent in systemic plasma. Our findings also imply that the increased rate of active renin secretion from the experimental, denervated kidney induced by captopril is due primarily to an increase in the intrarenal activation of renin, rather than to altered peripheral conversion of inactive to active renin (32). However, despite these rather clear-cut changes in renin secretion at the denervated experimental kidney, captopril did increase the total as well as the active renin concentration in arterial plasma. Although this was not specifically investigated, there may have been an increase in total renin secretion from the intact kidney, perhaps due to a reflex response of the 23 mm Hg fall in MAP induced by captopril administration to these salt-depleted dogs (Table 1). Thus, by using the same preparation, we had found previously that infusion of the Ang II antagonist saralasin reduced MAP and led to activation of renal nerves and total renin secretion from the kidneys (1). These changes in renin activation were not clearly reflected in renal lymph. This could not be explained by exclusion of captopril from the lymphatic compartment because measurements of ACE activity in lymph showed clear-cut inhibition. Although captopril reduced the Ang II concentrations of renal lymph consistently, inhibition both of Ang II generation and of Ang I-induced renal vasoconstriction was clearly incomplete. Larger doses of ACE inhibitor drugs can effectively prevent Ang I-induced renal vasoconstriction (30).

During formation, condensation, and packaging, preprorenin is converted to active renin intracellularly (33,34). We proposed that there are distinct intracellular pools of renin linked to separate secretory pathways (34). If captopril preferentially stimulated the secretion of active renin, there should be a corresponding change in the rate of total renin secretion. In contrast, our results show that captopril did not change total renin secretion into plasma at the time at which it had stimulated the release of active renin substantially. Therefore, it seems more likely that captopril stimulated the intracellular conversion step. The data suggest the interesting possibility that short-term changes in Ang II concentration in the kidney regulate its own renal production by feedback inhibition of the intracellular conversion of inactive to active renin. However, we cannot exclude the possibility that the effects of captopril are due to other actions of the drug, such as increased formation of prostaglandins or kinins (35).

Studies in the juxtaglomerular rat nephron or the hydronephrotic rat kidney show that Ang II applied from the external surface produces dose-dependent constriction of the afferent and efferent vessels within the range of 1 to 100 pM (2,3). After allowing for ex vivo generation, the concentrations of Ang II measured in renal lymph in this study in the dog were approximately 300 pM. Accordingly, Ang II is present in an extracellular site in the kidney in concentrations that may exert a profound physiological effect on the renal vessels. Consistent with this was the large reduction in RVR induced by captopril. This
probably represented a reduction in the afferent and efferent arteriolar resistances because there was a large fall in the filtration fraction. Indeed, the fractional changes in RVR produced by captopril were related to the fractional changes in Ang II concentration in renal lymph, whereas with the number of dogs studied, there were no significant correlations with the fractional changes in Ang II concentration in renal venous plasma. Therefore, this study provides indirect evidence to support a hemodynamically important contribution of interstitial Ang II in maintaining RVR in the salt-depleted dog kidney.

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