Intrarenal secretion of renin in the dog: effect of furosemide

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The activity of the renin-angiotensin system has usually been studied by either infusing the peptide into the kidney (12) or determining changes in plasma renin activity or renin secretion (15, 18). Thurau (19) has suggested that the rate of renin secretion into the systemic circulation may not reflect true intrarenal secretion of renin. However, data relating renal lymph angiotensin II concentration to renal hemodynamics suggest that the renin is secreted into the systemic circulation and into the renal interstitial space in a parallel manner (1).

Although several investigators have measured renin activity in renal lymph (9–11, 18), no systematic study has been undertaken to determine how changes in plasma renin activity and renal lymph renin activity are correlated. In addition, the renin activity in renal lymph has not been related to changes in renal function. The present studies were undertaken to further define the intrarenal action of the renin-angiotensin system. The results demonstrate that renin is secreted in parallel into both the renal interstitial space and the systemic circulation. Hemodynamic changes following stimulation of renin secretion by the diuretic, furosemide, cannot be explained by the pressor effect of angiotensin II on the renal vasculature.

METHODS

Experiments were carried out on male mongrel dogs anesthetized with intravenous pentobarbital sodium (30 mg/kg). After insertion of a cuffed endotracheal tube, all animals were artificially ventilated (Harvard Apparatus Co., Inc., respirator). Two catheters were placed in the aorta below the origin of the left renal artery via the femoral arteries in order to obtain arterial blood samples and monitor blood pressure by strain-gauge transducer (Statham P23AC). A femoral vein was cannulated for infusion of insulin and additional anesthetic agent. The left kidney was exposed through an extraperitoneal flank incision and both ureters identified and cannulated. Renal venous blood was obtained either from a polyethylene catheter passed into the left renal vein via the spermatic vein or from a curved 18-gauge needle placed directly into the renal vein and connected to polyethylene tubing. An electromagnetic flowmeter probe (Carolina Medical Electronics, Inc.) was placed on the left renal artery. A renal hilar lymph vessel was isolated and cannulated with polyethylene tubing.

Since the concentration of angiotensin II in renal lymph increases following a reduction in renal perfusion pressure and in association with autoregulation of renal hemodynamics, it seems unlikely that the pressor peptide plays a direct role in the mediation of the autoregulatory processes (1). Several investigators have suggested that in addition to its effect on renal hemodynamics, angiotensin II may directly modify other functions of the kidney including both proximal and distal tubular sodium reabsorption (12, 13).
(FF-50). Approximately 2 hr prior to starting the experiment, an intravenous infusion of inulin was started at a rate calculated to maintain arterial plasma levels at 25–30 mg/100 ml. A minimum of 1 hr was allowed for recovery of the animal following completion of surgery.

After obtaining one or two control samples of blood, lymph, and urine, the animals were given an intravenous infusion of isotonic saline (approximately 30 ml/kg) over 20 min to expand extracellular fluid volume and suppress renin secretion. The infusion rate was then reduced to 5 ml/min above urine output, and after urine flow had stabilized, two more samples each of blood, lymph, and urine were collected. The animals were then given furosemide (supplied by Hoechst Pharmaceuticals, Inc., Somerville, N. J.) intravenously and an intravenous infusion (1 mg/kg per hr) was started. In 10 animals, urinary losses were maintained by continued infusion of isotonic saline at 5 ml/min above urine flow rate. In five animals, the saline infusion was stopped following the injection of the diuretic. After urine flow had stabilized, two or three more samples were obtained.

Collection periods for blood, lymph, and urine ranged from 15 to 30 min in all experiments, and arterial and renal venous samples were continuously drawn into chilled plastic tubes containing disodium ethylenediaminetetraacetic acid (EDTA) as previously described (15).

Arterial and renal venous plasma and urine samples were analyzed for inulin using the diphenylamine method of Walser, Davidson, and Orloff (21). The concentration of angiotensin I in plasma and lymph was measured by radioimmunoassay as described by Haber et al. (7) using a renin activity radioimmunoassay kit (Schwarz/Mann). Sodium in plasma and urine was estimated by internal standard flame photometry (Eppendorf flame photometer). The hematocrit was determined on arterial blood by the micro method.

Renal blood flow (RBF) and mean systemic blood pressure were obtained from a direct-writing oscillograph recording (Öffner Dynograph). Glomerular filtration rate (GFR) was estimated from the clearance of inulin in the usual manner. Fractional sodium excretion was calculated as the clearance of sodium divided by the clearance of inulin. Renin activity in plasma and renal hilar lymph was estimated in the majority of periods in which values were high. The administration of furosemide resulted in large increases in lymph renin activity. Furthermore, the renin activity in lymph was greater than that in plasma in the majority of periods in which values were high (Fig. 4). During volume expansion, plasma and lymph

![Graph](http://ajplegacy.physiology.org/DownloadedFrom/10.2333/ajp01000426.10.2333/ajp01000426)

**Figure 1.** Mean values of plasma and lymph angiotensinogen concentration in 9 individual experiments. Bars show ± SEM. Line of identity is shown. P values are given for 3 animals in which plasma angiotensinogen concentration was greater than that in renal hilar lymph.

**Results**

**Angiotensinogen.** Figure 1 demonstrates the relationship between the concentration of angiotensinogen (renin substrate) in systemic arterial plasma and renal hilar lymph in 9 animals. While there was considerable variation between animals, there was a high correlation between the concentration of angiotensinogen in plasma and renal lymph ($r = 0.83$). The mean concentration of angiotensinogen in plasma was higher than that in lymph in three animals and was equal to that in the lymph in the remaining six. This finding is important since the renin activity in lymph was usually greater than that in plasma.

**Renin activity in plasma and lymph.** Since the response to furosemide administration in volume-expanded animals in both groups was similar, the data have been combined in Figs. 2 and 3. With the exception of one animal, plasma renin activity fell following volume expansion, while furosemide caused a rise in plasma renin activity (Fig. 2). Increases in renin activity in both plasma and lymph took place within 15 min of the injection of furosemide. In both groups of animals, volume expansion was maintained as indicated by the low hematocrit (Tables 1 and 2). Continued fluid losses in the animals not receiving replacement of urinary losses resulted in volume contraction and further increases in renin activity.

Volume expansion also suppressed renin activity in renal hilar lymph (Fig. 3). The administration of furosemide resulted in large increases in lymph renin activity. Furthermore, the renin activity in lymph was greater than that in plasma in the majority of periods in which values were high (Fig. 4).
TABLE 1. Effects of saline volume expansion and furosemide administration on renal hemodynamics in animals with replacement of urinary losses

<table>
<thead>
<tr>
<th>Condition</th>
<th>Exp No.</th>
<th>BP, mm Hg</th>
<th>GFR, ml/min per g</th>
<th>RBF, ml/min per g</th>
<th>RR, mm Hg</th>
<th>Hct, %</th>
<th>FNa, %</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>136</td>
<td>0.75</td>
<td>1.5</td>
<td>1.53</td>
<td>42</td>
<td>1.6</td>
</tr>
<tr>
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<td>145</td>
<td>0.40</td>
<td>2.4</td>
<td>1.01</td>
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<td>6.0</td>
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<td>41</td>
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<td>38</td>
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<td>36</td>
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<td>5.0</td>
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<td>3.4</td>
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<td>40.6</td>
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</table>

BP = mean blood pressure. GFR = glomerular filtration rate. RBF = renal blood flow. RR = renal resistance. Hct = hematocrit. FNa = fractional sodium excretion. PRU = peripheral resistance units.

renin activities tended to approach each other. Endogenous angiotensin I concentration was also found to be greater in the renal lymph than in the arterial plasma (Fig. 5).

While the rate of lymph flow was not measured in these experiments, observations of the volumes of lymph collected indicated that both volume expansion and furosemide administration produced increases in lymph flow rate.

Renal hemodynamics. Figures 6 and 7 show effects of volume expansion and furosemide on RBF, mean systemic blood pressure, GFR, and the renin-angiotensin system. In the

FIG. 2. Changes in plasma renin activity following saline volume expansion and furosemide administration. (C = control, S = saline volume expansion, F = furosemide.)

FIG. 3. Changes in renin activity in renal hilar lymph following saline expansion and furosemide administration. (C = control, S = saline expansion, F = furosemide.)
animal in which urinary losses were replaced, saline volume expansion resulted in a rise in RBF (Fig. 6) while no change was seen in the other animal (Fig. 7). GFR rose in both experiments during volume expansion. In renal lymph, endogenous angiotensin I concentration and renin activity fell as did plasma renin activity. Following furosemide, RBF increased and GFR fell. Renin activity in plasma and lymph rose as previously described. Experiment 203 (Fig. 6) was the only animal in which lymph renin activity was clearly less than plasma renin activity in the control periods.

Tables 1 and 2 summarize the hemodynamic data on the remaining animals. In addition to the fall in plasma and lymph renin activity, volume expansion resulted in an increase in GFR in all animals except one (experiment 218). Changes in renal resistance were more variable. In some animals, blood pressure rose (experiments 201, 202, 207, and 218) and with the exception of experiment 207, resistance rose. In other animals, the pressure changes were small during volume expansion and renal resistance was relatively constant. These variable changes in resistance took place in the face of a fall in renin activity in plasma and lymph in all animals.

Following furosemide, renal resistance fell in all animals in which fluid losses had been replaced (Table 1 and Fig. 6). The changes were more variable in those animals not receiving additional fluid replacement (Table 2), decreasing in two animals, increasing in two, and remaining constant in
one. GFR fell in both groups in all experiments except two (experiments 202 and 203).

In neither group was the state of volume expansion significantly altered within the first 30 min after furosemide, as indicated by the hematocrit values. Sodium excretion increased following volume expansion and furosemide as expected.

**DISCUSSION**

The present experiments demonstrate that renin activity in systemic plasma and renal hilar lymph can be modified in parallel. Volume expansion with isotonic saline suppressed both plasma and lymph renin activity while furosemide administration resulted in a parallel increase (Figs. 2 and 3). Renin has been previously reported to be present in renal lymph (9–11) and Lever and Peart (11) suggested that secretion of renin into lymph may be related to activity of the renin-angiotensin system within the kidney.

Thurau (19) has suggested that renin secretion into the systemic plasma may not reflect the secretion of renin into the renal interstitium. A recent report by Schmid (17) indicates that secretion of renin into the renal vein did not increase significantly during autoregulation of GFR and RBF following reduced renal perfusion pressure. In conjunction with the previous finding of elevated angiotensin II levels in lymph during autoregulation, the data of Schmid could support Thurau’s suggestion that intrarenal secretion of renin may not be reflected by changes in systemic secretion. However, the present studies indicate that systemic and intrarenal secretion of renin takes place simultaneously.

The concentration of angiotensin I and activity of renin are higher in renal lymph than in systemic arterial plasma (Figs. 4 and 5). Horkey et al. (10) demonstrated increases in lymph renin concentration following furosemide administration to rats, but the concentration of renin in lymph was less than that in aortic or renal venous plasma. The differences between the present data and those of Horkey et al. (10) may be explained by the fact that they were unable to obtain pure renal lymph in rats. The higher concentrations of renin, angiotensin I, and angiotensin II in renal lymph may be due to the smaller volume of renal interstitial fluid as compared to the systemic plasma volume.

Both Meyer et al. (14) and Vander and Carlson (20) have demonstrated that furosemide stimulates renin release independently of volume contraction. The former investigators found that volume expansion plus replacement of urinary losses by ureterovenous diversion prevented the rise in plasma renin activity following furosemide in rabbits (14). In the present experiments in the dog, volume expansion failed to inhibit the response to furosemide. Renin activity consistently rose after furosemide in spite of previous suppression by expansion of extracellular fluid volume. The data of Vander and Carlson (20) are consistent with the hypothesis that furosemide stimulates renin release by inhibition of sodium transport at the macula densa of the distal tubule. While this is a likely mechanism in the present experiments, the fall in GFR frequently seen following furosemide might also be important.

In the present studies, RBF consistently rose and GFR frequently fell after furosemide. Thus, renal resistance decreased in a manner suggesting efferent arteriolar vasodilatation. In view of the increased RBF and decreased total renal resistance following furosemide, in association with the increased intrarenal renin secretion, it is unlikely that the renin-angiotensin system is mediating this response.

The mechanism by which furosemide causes an increase in RBF may be due to a direct effect of the drug or an increase in intratubular pressure similar to the response seen with ureteral obstruction. Increased intratubular pressure, and a reduction in effective filtration pressure in volume-expanded animals, might explain the fall in GFR which is not seen with volume replacement alone (4, 14, 20).

The intrarenal action of the renin-angiotensin system remains unclear. It may be that the system plays a role in control of tubular function by mediating redistribution of renal blood flow under various conditions. Redistribution of renal blood flow has been demonstrated following both volume expansion and furosemide administration in the dog (3). However, further studies will be required to demonstrate the appropriate changes in angiotensin II concentration correlated with redistribution under various conditions. The present evidence does indicate that the renin-angiotensin system is active within the renal interstitium.

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**REFERENCES**


