Renin Release

RELATION TO RENAL SODIUM LOAD AND DISSOCIATION FROM HEMODYNAMIC CHANGES

By Franklin D. Nash, M.D., Howard H. Rotorfer, Ph.D., Michael D. Bailie, M.D., Ph.D., Ronald L. Wathen, Ph.D., and Edward G. Schneider, Ph.D.

ABSTRACT

The relations between renin release and renal hemodynamics, renal sodium load, and sodium and water balance were studied during hyponatremia, hypernatremia, mercurial natriuresis, norepinephrine infusion, and reduction in renal perfusion pressure. Significant reciprocal relations were found between renin secretory activity and the renal arterial plasma sodium concentration, the filtered sodium load, and urinary sodium excretion; however, no significant, or even consistent, relations were found between renin release and any hemodynamic parameter or sodium or water balance. During bilateral stimulation of renin release, unilateral restoration of the filtered sodium load inhibited ipsilateral renin release, without demonstrable changes in hemodynamics, while renin release from the contralateral kidney continued unabated. However, this inhibition was not seen unless the filtered sodium load and urinary sodium excretion rose to control levels even though renal venous plasma sodium concentrations exceeded 170 mEq/liter. Thus, renin release can be dissociated from hemodynamics and sodium and water balance, but not from some function of the filtered sodium load. We propose that renin secretory activity is controlled by an intrarenal, but extravascular, sodium-sensitive mechanism and that the stimulus is a function of the sodium flux across the macula densa into the interstitium surrounding the contiguous juxtaglomerular cells.

ADDITIONAL KEY WORDS control of renin secretion macula densa sodium and water balance hyponatremia hypernatremia organomercurial natriuretics norepinephrine renal perfusion pressure juxtaglomerular cells dogs

These studies were designed to evaluate the relative roles played by renal perfusion pressure, fluid balance, and renal sodium load in the regulation of renin release by the canine kidney (see reference 1 for review). In three series of experiments renal sodium load was dissociated from existing renal and systemic hemodynamics and from sodium and water balance. In the first we compared the effects of acutely induced hyponatremia and hypernatremia, alone and in conjunction with mercurial natriuresis, on renin release in dogs in positive sodium and water balance. In the second we studied the effects of unilateral restoration of renal sodium load on renin release by both kidneys of volume-expanded, hyponatremic dogs. In the third series we determined the effects of unilateral renal arterial hypernatremia on renin release induced by systemic and renal arterial infusion of norepinephrine and by reduction in renal perfusion pressure.
Methods

GENERAL ANIMAL PREPARATION

These experiments were carried out in male mongrel dogs weighing 14 to 28 kg. They were anesthetized with pentobarbital sodium (30 mg/kg intravenously) and maintained on intermittent positive-pressure respiration following endotracheal intubation. A double-lumen catheter was passed into the infrarenal aorta for sampling arterial blood and for recording renal perfusion pressure by a strain-gauge transducer. A peripheral vein was catheterized for infusions. When unilateral observations were made, the left kidney was approached through an extraperitoneal flank incision, and the ureter and renal vessels were exposed. A catheter was inserted into the ureter and connected to a conductivity cell and a photoelectric drop counter. An 18T-gauge needle was placed into the renal vein for continuous withdrawal of blood samples. A 24-gauge needle was inserted into the renal artery and connected to a motor-driven infusion pump. During control and recovery periods, isotonic sodium chloride was infused into the renal artery at 0.76 ml/min; this increased renal sodium delivery by only 0.1 mEq/min and did not alter renal venous plasma sodium concentrations. Renal arterial hypernatremia was produced by increasing the sodium chloride concentration of the infusate while maintaining the same rate of flow. A solution of creatinine and p-aminohippurate (PAH) in isotonic sodium chloride was administered intravenously at 0.5 ml/min; creatinine and PAH concentrations were calculated to maintain arterial plasma concentrations at 15 to 25 mg/100 ml and 1-2 mg/100 ml respectively. A heat exchanger was used to maintain systemic infusates at 38.5°C.

All experiments were clearance-extraction studies. The animals were heparinized, and arterial and renal venous blood samples were continuously drawn into chilled containers using a multichannel pump; flow rates and transit times in the blood sample lines were identical. Blood and urine collection periods were synchronous; since the contents of corresponding blood and urine samples were integrated over the same clearance period, analyses yielded representative mean values. Experimental periods were bracketed by control and recovery periods. In some experiments the blood sampling periods were of one minute's duration in order to demonstrate transient responses in renal venous and arterial plasma renin concentrations.

ANALYTICAL METHODS AND DATA HANDLING

The outputs of the pressure transducers, conductivity cells, and photoelectric drop counters were recorded with Beckman/Offner Dynographs. In the two series of experiments involving rapid systemic infusions, renal arterial inflow (non-cannulating electromagnetic flowmeter probe on the renal artery), central venous pressure, heart rate (cardiotachometer), and rectal temperature were also recorded.

A four-channel Technicon AutoAnalyzer system was used to determine the concentrations of creatinine, PAH, sodium, and potassium in plasma and urine using a modification of the simultaneous colorimetric method described by Harvey and Brothers (2) and by flame photometry using lithium as an internal standard. The hematocrit of each blood sample was determined in duplicate using the capillary tube method.

Renin concentrations in plasma were determined by bioassay as previously described (3). After treatment to remove preformedpressor substances and to inactivate angiotensinases, the samples were incubated to permit formation of angiotensin by the activity of renin on endogenous renin substrate. The blood pressure responses of the nephrectomized rat, treated with dibenzylene and ansolysin, were used to estimate the renin activity of the samples. Renin activity was expressed as nanograms of angiotensin equivalents per milliliter (ng/ml), and no attempt was made to express the results in Coldblatt units, as in earlier work (3).

Creatinine clearance was taken as a measure of glomerular filtration rate. Total renal plasma flow was calculated from the clearance and extraction of PAH in experiments in which the urine flow was below 2.0 ml/min; when urine flow was in excess of 2.0 ml/min, Wolf's formula (4) was used. Renal blood flow was calculated from the renal plasma flow and the hematocrit. GFR and renal plasma and blood flows were expressed as milliliters per minute per gram of kidney weight (ml/min x g). Sodium excretion was expressed either in absolute terms, as microequivalents per minute per gram of kidney weight (µEq/min x g), or as fractional sodium excretion, the percent of the filtered load excreted. Equivalent renin concentrations in renal venous and systemic arterial plasma and the renal venous-systemic arterial (V-A) plasma renin difference were expressed as nanograms of angiotensin equivalents per milliliter of plasma (ng/ml). In experiments in which urine flows were low, renin release (RR) was calculated as the product of the renal plasma flow (RPF) and the V-A plasma renin difference (RR = RPF × (V-A)Plasma). When urine flow was high, the following formula was used to compensate for concentration of renin in the renal venous plasma due to urinary water loss, RR = [(RPF - Vv) × (VvPlasma) - (RPF) × (APlasma)]. Renin release was expressed as nanograms of...
TABLE 1

Effects of Normonatremic Volume Expansion on Renin Release and Renal Function in Five Sodium- and Water-Loaded Dogs (Group A)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Periods</th>
<th>RR (ng/min • g)</th>
<th>GFR (ml/min • g)</th>
<th>RBF (ml/min • g)</th>
<th>AVn (mEq/liter)</th>
<th>FSE (%)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-0</td>
<td>1-4</td>
<td>0.85 ± 0.38</td>
<td>0.77 ± 0.04</td>
<td>3.79 ± 0.23</td>
<td>146 ± 0.94</td>
<td>2.49 ± 0.18</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>0-80</td>
<td>5-8</td>
<td>0.79 ± 0.18</td>
<td>0.81 ± 0.04</td>
<td>3.39 ± 0.11</td>
<td>147 ± 0.81</td>
<td>2.49 ± 0.19</td>
<td>38 ± 1</td>
</tr>
<tr>
<td>60-120</td>
<td>9-12</td>
<td>0.85 ± 0.31</td>
<td>0.83 ± 0.04</td>
<td>3.71 ± 0.15</td>
<td>148 ± 0.71</td>
<td>3.38 ± 0.28</td>
<td>33 ± 1</td>
</tr>
<tr>
<td>120-180</td>
<td>13-16</td>
<td>0.80 ± 0.34</td>
<td>0.84 ± 0.04</td>
<td>3.91 ± 0.16</td>
<td>148 ± 0.73</td>
<td>3.28 ± 0.24</td>
<td>33 ± 1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 20. Infused: water, 2496 ± 107 ml; sodium, 362 ± 16 mEq; excreted: water, 1104 ± 156 ml; sodium, 117 ± 22 mEq. RR = renin release; GFR = glomerular filtration rate; RBF = renal blood flow; AVn = arterial plasma sodium concentration; FSE = fractional sodium excretion.

Results

SERIES 1. EFFECTS OF CHANGES IN RENAL SODIUM DELIVERY AND URINARY SODIUM EXCRETION ON RENIN RELEASE IN SODIUM- AND WATeR-LOADED DOGS

Twenty dogs were sodium and water loaded by infusion of 0.85% sodium chloride into the suprarenal aorta at 0.5 ml/min • kg body weight for a minimum of 60 minutes following completion of the surgical preparation. This was followed by four 15-minute control periods (60 to 0 minutes, periods 1 through 4) and then by twelve 15-minute experimental periods (0 to 180 minutes, periods 5 through 16). The four experimental plans used were designed to show the effects of changes in arterial plasma sodium concentration, renal sodium load, and urinary sodium excretion on renin release while renal hemodynamics were stable and positive sodium and water balance was maintained. In five control dogs the normonatremic volume expansion was continued through the three experimental hours (group A, Table 1). In five dogs the infusate was changed to 0.42% sodium chloride, at the same volume rate, during the experimental periods to produce a hyponatremic volume expansion and decrease in renal sodium delivery (group B, Table 2). Six dogs received the 0.42% sodium chloride during the experimental periods and, in addition, were given 100 mg of mercury, as mercaptomerin, intravenously to assure an increase in urinary sodium excretion concomitant with the development of hyponatremia and reduction in renal sodium load (group C, Table 3). In four additional dogs the normonatremic loading and control periods were followed by infusion of 2.0% sodium chloride at the same volume rate and 100 mg of mercury given intravenously to produce increases in the systemic arterial plasma sodium concentration and renal sodium delivery concomitant with an increase in urinary sodium excretion (group D, Table 4).

With continued normonatremic volume expansion (group A, Table 1), there were no significant changes in renin release, renal blood flow, or the arterial plasma sodium concentration. Glomerular filtration rate and fractional sodium excretion increased (P<0.05), while the hematocrit decreased (P<0.02). During the 4 hours of observation, the animals excreted 44% (range, 28-53%) of the administered water and 32% (22-45%) of the sodium.

Hyponatremic volume expansion (group B, Table 2) was associated with an increase in
TABLE 2
Effects of Hyponatremic Volume Expansion on Renin Release and Renal Function in Five Sodium- and Water-Loaded Dogs (Group B)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Periods</th>
<th>RR (ng/min • g)</th>
<th>GFR (ml/min • g)</th>
<th>RBF (ml/min • g)</th>
<th>AV_ (mEq/liter)</th>
<th>FSE (%)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-0</td>
<td>1-4</td>
<td>1.53 ± 0.37</td>
<td>0.81 ± 0.02</td>
<td>5.46 ± 0.36</td>
<td>147 ± 0.65</td>
<td>3.36 ± 0.58</td>
<td>40</td>
</tr>
<tr>
<td>0-60</td>
<td>5-8</td>
<td>1.45 ± 0.39</td>
<td>0.83 ± 0.03</td>
<td>4.51 ± 0.25</td>
<td>146 ± 0.70</td>
<td>2.73 ± 0.41</td>
<td>37</td>
</tr>
<tr>
<td>60-120</td>
<td>9-12</td>
<td>1.34 ± 0.26</td>
<td>0.89 ± 0.04</td>
<td>4.40 ± 0.47</td>
<td>145 ± 0.70</td>
<td>1.75 ± 0.16</td>
<td>35</td>
</tr>
<tr>
<td>120-180</td>
<td>13-16</td>
<td>0.79 ± 0.24</td>
<td>0.83 ± 0.01</td>
<td>4.46 ± 0.34</td>
<td>139 ± 0.32</td>
<td>1.32 ± 0.12</td>
<td>34</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n = 20. Infused: water, 2592 ± 131 ml; sodium, 236 ± 12 mEq; excreted: water 890 ± 91 ml; sodium, 76 ± 20 mEq.
Abbreviations as in Table 1.

TABLE 3
Effects of Hyponatremic Volume Expansion plus Mercurial Natriuresis on Renin Release and Renal Function in Six Sodium- and Water-Loaded Dogs (Group C)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Periods</th>
<th>RR (ng/min • g)</th>
<th>GFR (ml/min • g)</th>
<th>RBF (ml/min • g)</th>
<th>AV_ (mEq/liter)</th>
<th>FSE (%)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-0</td>
<td>1-4</td>
<td>1.21 ± 0.30</td>
<td>0.81 ± 0.03</td>
<td>4.43 ± 0.24</td>
<td>147 ± 0.30</td>
<td>3.57 ± 0.30</td>
<td>38</td>
</tr>
<tr>
<td>0-60</td>
<td>5-8</td>
<td>2.45 ± 0.60</td>
<td>0.82 ± 0.03</td>
<td>4.41 ± 0.20</td>
<td>146 ± 0.46</td>
<td>6.06 ± 0.62</td>
<td>38</td>
</tr>
<tr>
<td>60-120</td>
<td>9-12</td>
<td>4.05 ± 0.66</td>
<td>0.77 ± 0.04</td>
<td>4.28 ± 0.13</td>
<td>141 ± 0.52</td>
<td>8.83 ± 0.43</td>
<td>39</td>
</tr>
<tr>
<td>120-180</td>
<td>13-16</td>
<td>7.01 ± 0.94</td>
<td>0.70 ± 0.34</td>
<td>3.75 ± 0.16</td>
<td>130 ± 0.53</td>
<td>6.89 ± 0.34</td>
<td>39</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n = 24. Infused: water, 2560 ± 139 ml; sodium, 233 ± 10 mEq; excreted: water 1623 ± 323 ml; sodium, 211 ± 22 mEq.
Abbreviations as in Table 1.

Renin release that was significant when compared with the control rates for that group (P < 0.001) and with the corresponding periods in group A (P < 0.001). Renal blood flow was lower than control during all the experimental hours (P < 0.05), but glomerular filtration rate did not change. Both the sodium concentration in arterial plasma and the fractional sodium excretion decreased (P < 0.001 and P < 0.01). The hematocrit declined (0.05 > P > 0.01), although the change was not significantly different from that which occurred in group A, and the animals excreted 34% (28-39%) of the infused water and 31% (7-49%) of the sodium.

In the animals that received mercaptomerin in addition to the hyponatremic infusion (group C, Table 3), renin release increased to levels significantly greater than the control values (P < 0.001), and the values for the corresponding periods in group A (P < 0.001), but they were not significantly different from the levels reached in group B. This is of particular interest since the animals in group C excreted 63% (57-98%) of the infused water, twice the proportion excreted by those...
TABLE 4
Effects of Hypematremic Volume Expansion plus Mercurial Natriuresis on Renin Release and Renal Function in Four Sodium- and Water-Loaded Dogs (Group D)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Periods</th>
<th>RR (ng/min • g)</th>
<th>GFR (ml/min • g)</th>
<th>RBF (ml/min • g)</th>
<th>ANi (mEq/liter)</th>
<th>PSE (%)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-0</td>
<td>1-4</td>
<td>±0.39</td>
<td>±0.14</td>
<td>±0.32</td>
<td>±0.68</td>
<td>±0.50</td>
<td>±1</td>
</tr>
<tr>
<td>0.85% Sodium Chloride, 0.5 ml/(min • kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-60</td>
<td>5-8</td>
<td>±0.47</td>
<td>±0.04</td>
<td>±0.28</td>
<td>±0.92</td>
<td>±0.77</td>
<td>±1</td>
</tr>
<tr>
<td>2.0% Sodium Chloride, 0.5 ml/(min • kg), + 100 mg Hg iv</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60-120</td>
<td>9-12</td>
<td>±0.34</td>
<td>±0.06</td>
<td>±0.45</td>
<td>±0.41</td>
<td>±0.92</td>
<td>±1</td>
</tr>
<tr>
<td>120-180</td>
<td>13-16</td>
<td>±0.37</td>
<td>±0.05</td>
<td>±0.41</td>
<td>±1.25</td>
<td>±0.37</td>
<td>±2</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n = 16. Infused: mater, 2520 ± 139 ml; sodium, 1615 ± 89 mEq; excreted: water, 2451 ± 174 ml; sodium, 512 ± 43 mEq.
Abbreviations as in Table 1.

TABLE 5
Significance of Weighted Mean Values (t) for Homogeneous Correlation Coefficients in Series I Experiments

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedure</th>
<th>n</th>
<th>df</th>
<th>((\text{RR vs. ANi}))</th>
<th>((\text{RR vs. FNa}))</th>
<th>((\text{RR vs. PSE}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>(\downarrow)Na</td>
<td>80</td>
<td>66</td>
<td>-0.820</td>
<td>-0.315*</td>
<td>-0.412†</td>
</tr>
<tr>
<td>C</td>
<td>(\downarrow)Na + Hg</td>
<td>96</td>
<td>79</td>
<td>-0.630†</td>
<td>-0.655†</td>
<td>-0.104</td>
</tr>
<tr>
<td>B + C</td>
<td></td>
<td>176</td>
<td>144</td>
<td>-0.655†</td>
<td>-0.520†</td>
<td>-0.258*</td>
</tr>
</tbody>
</table>

*P < 0.05. †P < 0.001.
FNa = filtered sodium load. df = \(n' - 2\), where \(n' = (b) + (a-1) (b-3)\), and a = the number of experiments and b = the number of observations in each experiment. Other abbreviations as in Table 1.

In group B \((P < 0.01)\), and showed no change in the hematocrit, again different from group B \((P < 0.01)\), although the animals in both groups received the same infusate at the same volume rate on the basis of body weight. Furthermore, in group C there was an increase in fractional sodium excretion \((P < 0.001)\); 90% (74-117%) of the sodium administered was excreted; and the decrease in sodium concentration in arterial plasma was greater than in group B \((P < 0.05)\). Therefore, although there were significant differences in sodium and water balance and both absolute and fractional sodium excretion between the two groups receiving the hyponatremic infusate, these variables did not appear to influence the increase in renin secretory activity that occurred during acutely induced hyponatremia.

In contrast with the data from groups B and C are those obtained during hypematremic volume expansion with superimposed mercurial natriuresis (group D, Table 4). These animals excreted 97% (88-102%) of the infused water and 32% (27-39%) of the infused sodium. There was no significant change in renin release as compared with the control values or with those of group A. Renin secretory activity in group D was significantly lower \((P < 0.001)\) than in group C, although the latter excreted a significantly smaller fraction of the infused water \((0.05 > P > 0.01)\), and both groups received the same amount of water and same dose of mercaptomerin.
The correlation coefficients between renin release and each of the other variables measured were determined for every experiment. The values derived for each relationship within a given group were tested for homogeneity, and if they were found to be homogeneous, a weighted mean value (?/?) was determined for the group and then tested for significance (6). The correlation coefficients between renin release and renal perfusion pressure, renal blood flow, and glomerular filtration rate were found to be heterogeneous in all groups, an indication of no significant, or even consistent, relationships. The correlation coefficients between renin release and sodium concentration in arterial plasma, filtered sodium load, and fractional sodium excretion were found to be heterogeneous for groups A and D; however, they were homogeneous within and between groups B and C. As indicated by the summary in Table 5, there were significant inverse relationships between renin release and both sodium concentration in arterial plasma and filtered sodium load.

**SERIES II. EFFECTS OF UNILATERAL RESTORATION OF RENAL SODIUM LOAD ON RENIN RELEASE DURING ACUTELY INDUCED HYPONATREMIA**

In five dogs arterial hyponatremia was induced by infusion of 0.42% sodium chloride into the suprarenal aorta at 0.5 ml/min·kg body weight. After the sodium concentration in systemic arterial plasma had fallen to 135 mEq/liter, there were four 15-minute control periods. The infusion rate of sodium chloride into the left renal artery was then increased to restore the plasma sodium concentration in the ipsilateral renal artery to normonatremic levels for four periods, following which the infusion rate was reduced to the control rate for four additional periods. The rate required to achieve unilateral renal arterial normonatremia for each experiment was estimated from the directly measured renal arterial blood flow, the hematocrit value, and the sodium concentration in systemic arterial plasma. In two of the dogs in this series, samples were collected synchronously from both renal veins, both ureters, and the infrarenal aorta so that data on renin release and renal function could be obtained for both kidneys.

In all five animals renin secretory activity was high during the hyponatremic control periods. When the concentration of the sodium chloride infusate in the left renal artery was increased, plasma sodium concentration in the ipsilateral renal vein, filtered sodium load, and fractional sodium excretion increased, and renin release decreased (0.01 >
RENAL SODIUM LOAD AND RENIN SECRETION

FIGURE 2

Effects of renal arterial hypernatremia on renin release and renal function in two control dogs. In A the control renin release was within 1 SD of the pooled control value in 31 similarly prepared dogs; in B the control renin release was four times greater than 1 SD of the pooled value. FA = femoral artery.

$P > 0.001$). Subsequent reduction of the renal arterial sodium chloride infusion to control levels was accompanied by decreases in the sodium concentration in renal venous plasma, filtered sodium load, and fractional sodium excretion; renin release rose to the control levels.

Figure 1 illustrates the results obtained in one of the two experiments in this series in which bilateral measurements were made. It is apparent that the increases in sodium concentration in renal venous plasma and fractional sodium excretion and the decrease in renin secretory activity during the periods of increased left renal arterial sodium delivery were restricted to the ipsilateral organ, while renin release by the right kidney, which remained hyponatremic, was unaltered.

In this series of experiments, as in groups B and C of series I, renin release was inversely related to the sodium concentration in renal venous plasma, fractional sodium excretion,
FIGURE 3

Stimulation of renin release by fall in arterial blood pressure (ABP) following termination of intravenous infusion of norepinephrine at 16 μg/min (A); inhibition of this effect by renal arterial hypernatremia (B). The index of dispersion is 1 so.

and the estimated sodium concentration in renal arterial plasma and filtered sodium load. There was no consistent relationship between renin release and glomerular filtration rate, renal blood flow, renal perfusion pressure, sodium or water balance, or sodium concentration of systemic arterial plasma when the latter was different from the sodium concentration in renal arterial plasma.

SERIES III: EFFECTS OF RENAL ARTERIAL HYPERNATREMIA ON RENIN RELEASE INDUCED BY NOREPINEPHRINE INFUSION AND BY REDUCTION IN RENAL PERFUSION PRESSURE

A. Control.—In two experiments, two 15-minute control periods were followed by four
periods during which the renal arterial sodium chloride infusion was increased from 0.1 mEq/min to 1.6 mEq/min. No other manipulation was performed. As seen from Figure 2, renal arterial hypematremia alone had little effect on glomerular filtration rate or renal plasma flow, although urinary sodium excretion increased. Renin release was depressed during the period of renal arterial hypematremia and increased sodium excretion, regardless of the control rate of renin secretion, and was unrelated to renal hemodynamics.

B. Reduction in Perfusion Pressure Following Termination of Intravenous Norepinephrine Infusion.—In seven dogs, following completion of a 30-minute control period, norepinephrine was infused intravenously at 16 μg/min for 30 minutes. In three of the dogs, the norepinephrine infusion was stopped and the effect of the subsequent fall in arterial pressure on renin release was observed over a 30-minute period. In the other four, the renal arterial sodium chloride infusion was increased from 0.1 to 1.3 mEq/min, synchronous with the termination of the norepinephrine infusion, and the responses were followed for 30 minutes. The results of these two groups of experiments are summarized in Figure 3.

During the period of norepinephrine infusion, there were no significant differences between the two groups in regard to any of the variables examined. Upon termination of the norepinephrine infusion in the normonatremic groups (Fig. 3, A), the fall that occurred in arterial pressure was accompanied by increases in the V-A plasma renin difference (P < 0.001) and in renin secretory activity (P < 0.05), and there was a decrease in urinary sodium excretion. In contrast, in the hypeniatremic group (Fig. 3, B) urinary sodium excretion increased during the period of reduced perfusion pressure, and there was no increase in renin release. In the period following termination of the norepinephrine infusion, the V-A plasma difference and the rate of renin release were significantly higher (P < 0.001 for both) in the normonatremic animals than in those in which renal sodium delivery was increased, but there were no significant differences between the two groups in regard to arterial pressure, glomerular filtration rate, or renal plasma flow.

In four additional dogs, a similar procedure was followed, except that norepinephrine was infused for only 15 minutes, and renal venous and systemic arterial blood samples were drawn over 1-minute intervals during the last 2 minutes of the norepinephrine infusion period and during the first 7 minutes following termination of the norepinephrine infusion and the simultaneous onset of renal arterial hypematremia. Figure 4 illustrates the time course of the changes in
the content of renin in arterial and renal venous plasma and the V-A plasma renin difference under these circumstances. Immediately upon termination of the norepinephrine infusion, renin activities in arterial and renal venous plasma and the V-A plasma renin difference increased coincidentally with a rapid decline in arterial blood pressure. By the fifth minute of the response, the V-A plasma renin difference had fallen below its control level, and it remained low throughout the remainder of the period; sodium concentration in ipsilateral renal venous plasma and urinary sodium excretion were elevated. It is important to point out that the renin content of systemic arterial plasma rose during the periods of rapid sampling and remained elevated thereafter. The increase in the V-A plasma renin difference for the experimental kidney was transient, and its estimated renin release can account for no more than 12% of the observed increase in the level of renin in arterial plasma (3). Therefore, it is probable that the high and sustained renin concentration in arterial plasma was a reflection of a marked increase in renin release from the contralateral (nor-

![Figure 5](http://circres.ahajournals.org/)

**Figure 5**

*Effects on renin release and renal function of renal arterial infusion of norepinephrine both with and without concurrent increases in renal sodium load.*

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monatremic) kidney in response to the fall in arterial blood pressure, and that renin secretory activity by the experimental (hypernatremic) kidney was depressed.

C. Renal Arterial Infusion of Norepinephrine.—In three dogs, renin release was stimulated by renal arterial infusion of norepinephrine in 0.9% sodium chloride at 3.0 μg/min and 0.1 mEq/min (3.0/0.1 infusion). The responses obtained were compared in the same animals to those produced by infusion of norepinephrine in 10% sodium chloride at rates of 3.0 μg/min and 1.3 mEq/min (3.0/1.3 infusion). In two of the experiments, the 3.0/0.1 infusion preceded the 3.0/1.3 infusion. In the third (Fig. 5), the sequence was reversed to demonstrate that the difference in the responses to the two infusates was not due to exhaustion of the renin release mechanism. Rapid sampling revealed the time courses of the responses.

During the 3.0/0.1 infusion, there were rapid and progressive increases in the renal venous and systemic arterial plasma renin concentrations and in the V-A plasma renin difference, and they remained elevated throughout the entire period. Urinary sodium excretion, renal plasma flow, and glomerular filtration rate were reduced. In contrast, although the 3.0/1.3 infusion resulted in similar reductions in renal plasma flow, the renal venous plasma sodium concentration and urinary sodium excretion increased, and the increase that occurred in the V-A plasma renin difference was smaller and less sustained. It is unlikely that the blunted renin release response to the 3.0/1.3 infusion, as compared to that produced by the 3.0/0.1 infusion, was due to reduced biological activity of the norepinephrine when prepared in hypertonic sodium chloride, since the constrictor effect, as evidenced by the reduction in renal plasma flow, was comparable in these experiments regardless of the concentration of sodium chloride in the infusate.

In three other experiments, the same sequence was followed, but the infusion rate of norepinephrine was higher, 4.0 μg/min in both the normonatremic and hypernatremic infusions. Renal arterial hypernatremia did not depress the renin release response to the higher dose of norepinephrine. Renin release averaged 5.59 ng/min • g during the 4.0/0.1 infusion and 6.12 ng/min • g during the 4.0/1.3 infusion. During the 4.0/1.3 infusion there was no increase in urinary sodium excretion despite the fact that sodium concentrations in renal venous plasma rose to over 165 mEq/liter. Renal plasma flow and glomerular filtration rate were reduced to 50% of their respective control values.

D. Reduction in Perfusion Pressure.—In five experiments, renal perfusion pressure was reduced by controlled suprarenal aortic compression during each of three 15-minute periods (periods 2, 4, and 6 in Table 6). During period 4, renal sodium delivery was elevated to 2.6 mEq/min by increasing the sodium chloride concentration of the renal arterial infusate 90 to 120 seconds prior to the onset of pressure reduction. The data from four experiments are summarized in Table 6; qualitatively similar data were obtained in the fifth experiment, but it was omitted from the statistical analysis due to an unusually high rate of renin release during the control period.

In each of the three periods of pressure reduction the decreases in perfusion pressure, glomerular filtration rate, and renal plasma flow were comparable and not significantly different. During the initial normonatremic pressure reduction (period 2), there was a significant increase in renin release (P < 0.01). In contrast, during the hypernatremic pressure reduction (period 4), renin release was significantly lower than the rates in the immediately preceding period (P < 0.01) and in the initial (P < 0.001) or subsequent (0.5 > P > 0.01) periods of normonatremic pressure reduction. During the two periods of normonatremic pressure reduction, urinary sodium excretion was depressed (P < 0.01); however, during the hypernatremic pressure reduction, the depression of renin secretory activity was associated with a rate of urinary sodium excretion which was greater than the preceding control values and significantly higher than
TABLE 6
Effects of Renal Arterial Hypernatremia on Renin Release and Renal Function during Reduction in Renal Perfusion Pressure Produced by Suprarenal Aortic Compression

<table>
<thead>
<tr>
<th>Period</th>
<th>RR (ng/min • g)</th>
<th>(V-A)renin (ng/ml)</th>
<th>VNa (mEq/liter)</th>
<th>UNaU (μEq/min)</th>
<th>GFR (ml/min • g)</th>
<th>RPF (ml/min • g)</th>
<th>ABP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.70 ± 4.33</td>
<td>4.75 ± 1.62</td>
<td>150</td>
<td>1.79</td>
<td>15.4 ± 1.21</td>
<td>0.78 ± 0.14</td>
<td>2.67 ± 0.38</td>
</tr>
<tr>
<td>2</td>
<td>15.4 ± 1.21</td>
<td>13.3 ± 1.21</td>
<td>147</td>
<td>0.68</td>
<td>11.6 ± 0.20</td>
<td>0.47 ± 0.08</td>
<td>66 ± 0.27</td>
</tr>
<tr>
<td>3</td>
<td>11.6 ± 0.20</td>
<td>11.6 ± 0.20</td>
<td>144</td>
<td>2.45</td>
<td>3.10 ± 0.81</td>
<td>0.97 ± 0.07</td>
<td>73 ± 0.17</td>
</tr>
<tr>
<td>4</td>
<td>3.10 ± 0.81</td>
<td>6.70 ± 2.42</td>
<td>175</td>
<td>2.90</td>
<td>5.40 ± 0.69</td>
<td>0.12 ± 0.13</td>
<td>13 ± 0.17</td>
</tr>
<tr>
<td>5</td>
<td>5.40 ± 0.69</td>
<td>5.30 ± 2.42</td>
<td>150</td>
<td>1.28</td>
<td>0.75 ± 0.07</td>
<td>0.47 ± 0.10</td>
<td>66 ± 0.19</td>
</tr>
<tr>
<td>6</td>
<td>6.70 ± 0.40</td>
<td>2.67 ± 0.38</td>
<td>150</td>
<td>1.28</td>
<td>6.70 ± 3.63</td>
<td>0.47 ± 0.30</td>
<td>132 ± 0.13</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of four observations. (V-A)renin = renal venous-systemic arterial plasma renin difference; VNa = renal venous plasma sodium concentration. UNaU = urinary sodium excretion; ABP = arterial blood pressure. Other abbreviations as in Table 1.

In 16 experiments in eight additional dogs, renal sodium delivery was not increased until 5 minutes after the onset of reduced renal perfusion pressure. Renal arterial hypernatremia was induced during the second 5 minutes of each of two 15-minute periods of pressure reduction. During the initial 5 minutes of reduced perfusion pressure, there was a sevenfold increase in the V-A plasma renin difference which was associated with a reduction of urinary sodium excretion to 36% of the control value. When the renal arterial sodium chloride infusion rate was then increased, there was no significant change in the rate of renin release; urinary sodium excretion rose to 66% of the control value, but did not reach control levels in any of the experiments, even though sodium concentrations in renal venous plasma were elevated above 170 mEq/liter in all the experiments.

Discussion
The results of these studies demonstrate that renin release can be dissociated from renal and systemic hemodynamics and from sodium and water balance but not from some intrarenal action of sodium. This dissociation was accomplished under a variety of experimental conditions and methods of stimulating renin release.

Vander and Luciano (7, 8) have suggested that the increase in renin release associated with mercurial natriuresis (9) and with the administration of other natriuretic agents (9, 10, 11) is due to salt and water depletion and subsequent stimulation of low-pressure extrarenal volume receptors (12, 13), the efferent limb being either the renal nerves or some unidentified humoral agent. The results of our series I and II experiments do not support this concept. In the former the three experimental hours were preceded by normonatremic volume expansion sufficient to suppress release of antidiuretic hormone (ADH) as determined by bioassay of systemic arterial blood (unpublished data). In the normonatremic group, ADH levels and renin release remained low; in the hyponatremic...
group, ADH levels remained low, but renin release increased. Since there was no reduction in plasma osmolality in the normonatremic group and since the hyponatremic animals excreted less of the infused water (34% vs. 44%) and exhibited a greater depression of the hematocrit (15% vs. 11%) than did the normonatremic dogs, it is probable that the degree of plasma volume expansion in the hyponatremic animals was sufficient to inhibit ADH release via a reduction in left atrial volume receptor activity (13, 14). Furthermore, the increases in renin release during hyponatremic volume expansion alone were not significantly different from those during hyponatremia with superimposed mercurial natriuresis, and in addition, they correlated inversely with the arterial plasma sodium concentration, filtered sodium load, and fractional sodium excretion. In contrast, there was no consistent relationship between renin release and any hemodynamic variable, sodium and water balance, or plasma volume as inferred from the hematocrit values. If, indeed, there was volume depletion and stimulation of extrarenal, or even intrarenal, volume receptor activity in any group, it should have occurred in the animals that received the hypernatremic infusate and mercaptomerin; nevertheless, there was no change in renin secretory activity in that group, although the animals excreted 97% of the infused water and ADH levels rose.

Evidence has been presented that organomercurials may not inhibit sodium reabsorption in the proximal tubule (15) and, in fact, may exert their inhibitory effect on sodium transport distal to the ascending limb of the loop of Henle (16). Thus, the postulated sodium receptor, the macula densa, would not be exposed to increased concentrations, or delivery rates, of sodium during mercurial natriuresis. If this is the case, the increased renin release in the group C experiments in series I would be explained solely on the basis of decreases in both the filtered sodium load and distal sodium delivery. The observed similarities between groups B and C would be anticipated as would the lack of stimulation of renin release in group D in which the filtered sodium load was increased in conjunction with the mercurial natriuresis. As attractive as this explanation is, it is not compatible with the reports that mercurial diuretics (17, 18), like chlorothiazide, acetazolamide, and mannitol (18), blunt the renin release response to reduction in renal perfusion pressure. Interpreted within the context of the macula densa hypothesis, the latter findings imply that the depression of renin release in those experiments was due to inhibition of sodium reabsorption proximal to the early distal tubule and a resulting increase in sodium delivery to the receptor. There is, however, an alternative explanation which is still consistent with the general hypothesis. It is possible that organomercurial diuretics inhibit sodium transport in all portions of the tubule, although the effects may be more marked in some segments than in others, so it is reasonable to assume that the sodium transport activity of the macula densa cells would also be reduced. With hyponatremia plus mercaptomerin, the delivery of sodium from the more proximal segments to the early distal tubule might have been increased, but there may also have been a reduction in net sodium transport across the macula densa. If the real stimulus to renin release is a function of the rate of delivery of sodium into the interstitium surrounding the juxtaglomerular cells, the combined effects of a reduction in filtered sodium load and partial inhibition of macula densa sodium transport would result in a stimulation of renin release. On the other hand, when plasma sodium levels were markedly elevated in conjunction with mercaptomerin administration, the increase in sodium delivery may have compensated passively for the reduction in macula densa transport ability so that there was no significant decrease in the net flux of sodium into the immediate environs of the juxtaglomerular cells and, therefore, no stimulus for increased renin release.

Although the foregoing is admittedly speculative, the reciprocal relationships observed between renin release and both the filtered sodium load and fractional sodium excretion,
and the lack of correlation between renin release and either hemodynamics or water balance, indicate that renin secretory activity is, in some way, inversely related to the rate of delivery of sodium to the tubules. The results of our experiments in hyponatremic, volume-expanded animals may be analogous to the observation that in patients with congestive heart failure renin concentrations in systemic plasma were generally inversely related to plasma sodium concentrations, but unrelated to arterial blood pressure, and that the plasma renin concentrations decreased during diuretic therapy (19). In those patients hypervolemia presumably would have precluded stimulation of renin secretion by volume receptors located in the atria or great veins.

The most direct evidence for a significant functional relationship between the filtered sodium load and renin release comes from the series II experiments. Unilateral restoration of the filtered sodium load in volume-expanded, hyponatremic animals inhibited ipsilateral renin release but had no effect on the contralateral kidney. It is highly improbable that this inhibitory effect could have been mediated via reflex mechanisms involving extrarenal volume or sodium receptors, and there is no indication that it was in any way related to renal or systemic hemodynamics or to sodium or water balance.

Finally, in the series III experiments sodium concentrations in renal venous plasma in excess of 170 mEq/liter were associated with a reduced renin release response only when filtered sodium load and urinary sodium excretion simultaneously increased to control levels. This suggests that the receptor involved in the dissociation of renin release from renal hemodynamics is responsive to some function of the filtered sodium load and not to the intravascular sodium concentration. Furthermore, if the necessity for initiating the renal arterial hypernatremia prior to the reduction in perfusion pressure is related to vascular and tubular transit times, it may well be that the receptor in question is intratubular.

In summary, the data obtained in these studies support the hypothesis that renin release is controlled by an intrinsic renal mechanism related to tubular sodium handling rather than to a primary baroreceptor function. Renin release was augmented by conditions which would be indicative of either sodium deficit or of loss and impending deficit, circumstances under which elevation of circulating angiotensin levels, stimulation of aldosterone secretion, and increased sodium and water retention would be appropriate homeostatic responses. We must emphasize that it is not as yet possible to define precisely either the nature of the stimulus or the identity of the receptor. If the macula densa is the receptor, the question still remains as to whether the controlling factor is the "flow or composition of the intratubular fluid" (18), a function of the net sodium flux into the interstitium surrounding the juxtaglomerular cells as suggested above, or, indeed, a specific effect of the sodium ion (20) as opposed to a non-specific osmotic action (21). Nevertheless, the evidence strongly indicates that renin release reflects the activity of an intrarenal sodium-conserving mechanism responsive to changes in glomerulotubular sodium balance.

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