Mechanism of the Effects of Furosemide on Renin Secretion in Anesthetized Dogs

By Arthur J. Vander, M.D., and Joyce Carlson

ABSTRACT
Furosemide was administered to anesthetized dogs in priming doses of 0.1, 0.5, or 2.5 mg/kg followed by the same amounts per hour as infusion. When sodium and water balances were maintained constant by the continuous replacement of urinary losses with isotonic saline, the renal venous renin activity (and renin release) was not altered by the 0.1 mg/kg dose but was significantly increased by the two larger doses. This increase was manifest within 5 to 10 minutes. The larger doses also increased mean arterial pressure but did not change plasma sodium or glomerular filtration rate. Renal plasma flow was increased by the 2.5 mg/kg dose but not by the 0.5 mg/kg. In another group of dogs in which salt depletion was allowed to occur, 0.1 mg/kg produced an increased renin release within 30 minutes; this increase was completely reversed by the replacement of sodium and water losses. In this group of dogs, the glomerular filtration rate decreased and then returned to control values after restoration of fluid balance. It is hypothesized that the two higher doses of furosemide stimulated renin release by directly inhibiting macula densa sodium transport, and that the reported effects of all natriuretics can be explained in terms of a single macula densa theory.

ADDITIONAL KEY WORDS angiotensin macula densa diuretics sodium reabsorption natriuretics renal hemodynamics

The effects of natriuretic drugs on renin secretion appear to be quite complex (see ref. 1 and Discussion). Previously, it was concluded (1-3) that these drugs stimulate renin secretion only when salt depletion is allowed to occur. However, recent studies with furosemide in rabbits (4) and with ethacrynic acid in dogs (5) have not been consistent with this conclusion. The present studies were designed to study this question further, using furosemide in dogs.

Methods
All experiments were performed on mongrel dogs weighing 14 to 22 kg and maintained on standard dog chow (Friskies). Animals were fasted overnight but were allowed free access to water. They were anesthetized with sodium pentobarbital, 30 mg/kg iv, with supplements given as required. Through a right flank incision, both ureters were catheterized and a polyethylene catheter (2.4 mm o.d.), which had been introduced into the left femoral vein and passed up the vena cava, was manipulated into the right renal vein. Renal venous blood was obtained from this catheter, and arterial blood was taken from a carotid artery catheter. Fluids, as described below, were administered via catheters in the brachial veins. Arterial pressure was monitored continuously from a femoral artery catheter using a Statham transducer and Grass polygraph. Renal excretory and hemodynamic data were obtained using standard clearance techniques. Clearance periods were 5 to 15 minutes long; arterial and renal venous blood samples were taken in the middle of the periods. After a priming dose of creatine and para-aminohippurate (PAH) was given intravenously, these substances were infused at a constant rate, 0.2 to 0.4 ml/min. Creatinine clearance was used as a measure of glomerular filtration rate, and total renal plasma flow was determined by the Fick principle, using PAH. In most experiments all red cells were suspended in an equal volume of 6% dextran-isotonic saline and returned to the dog. Experimental observations were not begun until at least 40 minutes after completion of all operative procedures and administration of the creatinine.
and PAH priming dose. Control clearances were then performed and one of two experimental protocols was followed.

Protocol 1.—After completion of control clearances, furosemide (Lasix) was administered intravenously to three groups of six dogs. Priming doses of 0.1, 0.5, or 2.5 mg/kg were followed by the same amounts per hour as infusion. Isotonic saline was then continuously administered intravenously at a rate equal to the urine flow. To accomplish this, the urine was collected in 50-ml graduate cylinders while, simultaneously, saline was injected manually from a 50-ml syringe. Each 50 ml collected or injected was recorded. As a double check, the 50-ml urine collections were pooled (after removal from each of 0.1 ml for sodium analysis) and the total volume compared to the total volume of saline administered. At no time in any experiment did volume loss differ from volume administered by more than 20 ml. Similarly, sodium balance at no time varied by more than 5 mM. Blood samples for renin were, in some cases, taken 5 minutes after beginning the furosemide, and during clearances performed 20 to 70 minutes later.

Protocol 2.—In 4 dogs, after completion of control clearances, a priming dose of furosemide, 0.1 mg/kg iv, was followed by an infusion of 0.1 mg/kg/hour. Thirty to 70 minutes later, additional clearances were performed. Isotonic saline was then infused rapidly (25 to 50 ml/min) in an amount equal to the total volume of urine excreted after furosemide administration began. After this "catch-up" period (approximately 10 minutes) the isotonic saline was continuously infused at a rate equal to the urine flow. Thirty minutes later, final clearances were performed.

Analytical Techniques.—Methods for sodium, PAH, creatinine, and renin analysis have been described previously (6). The only difference in the present method for renin was that 0.5 ml of 3.8% NH₄EDTA was added to samples before incubation as further protection against angiotensinases. Renin activity is expressed as nanograms of angiotensin equivalents generated during a 30-minute incubation per milliliter of plasma. In a series of in-vitro tests we found that the addition of exogenous renin (hog renin-NBC) caused the generation of identical amounts of angiotensin in plasma samples taken before and after furosemide administration. Therefore, the relationship between enzyme activity and enzyme concentration was not altered by furosemide; accordingly, in these experiments, changes in activity reflect changes in concentration. Finally, the changes in renal venous activity observed in these experiments adequately reflect changes in secretion rate since changes in renal plasma flow were either absent or very small (compared to the changes in renal venous renin).

Results

Protocol 1.—All data for protocol 1 are summarized in Figure 1 and Table 1. It should be re-emphasized that these studies were carried out without significant change in sodium or water balance.

The lowest dose of furosemide, 0.1 mg/kg, induced a small but significant natriuresis but no change in renin secretion, arterial pressure, or renal hemodynamics. The two higher doses induced progressively greater increases in sodium excretion and markedly stimulated renin release in every dog. The changes in renin induced by 0.5 and 2.5 mg/kg were not significantly different from each other. In five dogs, renal venous samples were obtained 5 to 10 minutes after beginning the furosemide and in every case renin was found to be already elevated. These two doses of furosemide also caused significant increases in mean arterial pressure, the time course being similar to that for the renin rise. Neither the 0.5 or 2.5 mg/kg dose altered the glomerular filtration
TABLE 1
Effects of Furosemide without Volume Depletion on Renal Function and Renin Release in Anesthetized Dogs

<table>
<thead>
<tr>
<th>Furosemide</th>
<th>0.1 mg/kg (n = 6)</th>
<th>0.5 mg/kg (n = 6)</th>
<th>2.5 mg/kg (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Renal venous renin (ng ang.-equiv./ml)*</td>
<td>-0.4 ± 0.1</td>
<td>7.6 ± 1.5</td>
<td>10.8 ± 2.0</td>
</tr>
<tr>
<td>A Mean arterial BP, mm Hg*</td>
<td>3.2 ± 3.0</td>
<td>7.2 ± 1.5</td>
<td>8.7 ± 2.5</td>
</tr>
<tr>
<td>A Plasma Na, mm*</td>
<td>0.0 ± 0.1</td>
<td>-0.3 ± 0.2</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>Percent filtered Na excreted (control &lt; 1%)</td>
<td>3.8 ± 1.0</td>
<td>14.5 ± 1.5</td>
<td>21.5 ± 3.3</td>
</tr>
<tr>
<td>Glomerular filtration rate, ml/min†</td>
<td>0.94 ± 0.03</td>
<td>1.12 ± 0.07</td>
<td>1.05 ± 0.09</td>
</tr>
<tr>
<td>Renal plasma flow, ml/min†</td>
<td>1.03 ± 0.06</td>
<td>1.09 ± 0.06</td>
<td>1.26 ± 0.09</td>
</tr>
<tr>
<td>Filtration fraction†</td>
<td>0.94 ± 0.07</td>
<td>1.05 ± 0.05</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td>Extraction ratio†</td>
<td>1.00 ± 0.02</td>
<td>1.03 ± 0.02</td>
<td>0.94 ± 0.03</td>
</tr>
</tbody>
</table>

The furosemide was given intravenously as a priming dose followed by a continuous infusion of the same quantity per hour. Fluid losses were continuously replaced with isotonic saline.

* Furosemide minus control. † Furosemide/control. § P = <.01. ¶ P = <.02. || P = <.05.

rate or PAH extraction ratio; the latter dose did, however, cause a significant increase in renal plasma flow and decrease in filtration fraction.

Protocol 2.—The experiments of protocol 2, in which salt depletion was allowed to occur and then remedied, are summarized in Table 2. In contrast to its effect when salt balance was maintained, 0.1 mg furosemide/kg caused renin secretion to increase in every dog when fluid losses were not replaced. Conversely, the restoration of fluid balance was associated

TABLE 2
Effects of Furosemide, 0.1 mg/kg, on Renal Venous Renin and Renal Function in Four Dogs

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Renal venous renin (ng ang.-equiv., ng/ml)</th>
<th>Negative Na balance (mg)</th>
<th>GFR (ml/min)</th>
<th>RPF (ml/min)</th>
<th>Plasma Na (mm)</th>
<th>Na excretion (nM/min)</th>
<th>Mean art. BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-10-0</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>156</td>
</tr>
<tr>
<td>Furosemide</td>
<td>-10-0</td>
<td>2.3</td>
<td>34.1</td>
<td>104</td>
<td>140</td>
<td>53</td>
<td>122</td>
</tr>
<tr>
<td>Fluid not replaced</td>
<td>30-40</td>
<td>4.3</td>
<td>22.8</td>
<td>28.0</td>
<td>111</td>
<td>141</td>
<td>140</td>
</tr>
<tr>
<td>Fluid replaced</td>
<td>50-60</td>
<td>5.9</td>
<td>30.0</td>
<td>26.1</td>
<td>114</td>
<td>141</td>
<td>87</td>
</tr>
<tr>
<td>Fluid replaced</td>
<td>60-70</td>
<td>1.2</td>
<td>36.0</td>
<td>124</td>
<td>141</td>
<td>464</td>
<td>130</td>
</tr>
<tr>
<td>Control</td>
<td>85-95</td>
<td>0.0</td>
<td>33.0</td>
<td>143</td>
<td>139</td>
<td>26</td>
<td>94</td>
</tr>
<tr>
<td>Furosemide</td>
<td>-10-0</td>
<td>2.7</td>
<td>41.6</td>
<td>231</td>
<td>140</td>
<td>21</td>
<td>143</td>
</tr>
<tr>
<td>Fluid not replaced</td>
<td>20-30</td>
<td>4.3</td>
<td>16.1</td>
<td>27.3</td>
<td>102</td>
<td>138</td>
<td>78</td>
</tr>
<tr>
<td>Fluid replaced</td>
<td>50-60</td>
<td>3.9</td>
<td>17.1</td>
<td>38.8</td>
<td>181</td>
<td>140</td>
<td>60</td>
</tr>
<tr>
<td>Fluid replaced</td>
<td>60-70</td>
<td>1.6</td>
<td>41.7</td>
<td>199</td>
<td>140</td>
<td>125</td>
<td>125</td>
</tr>
</tbody>
</table>

A priming dose of furosemide given intravenously at time zero was followed by continuous infusion of the same dose per hour. See Methods for further details. Except for negative Na balance, all renal data are for the right kidney only. Ang. equiv. = angiotensin equivalents; GFR = glomerular filtration rate; RPF = renal plasma flow.

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with a return of renin secretion to control values. During the period of salt depletion, arterial pressure and plasma sodium remained stable. In every dog, the glomerular filtration rate decreased and then returned to control values after restoration of fluid balance. Renal plasma flow showed similar changes in two of three dogs. Note that the replacement of fluid losses caused a large increase in sodium excretion, indicating that the full effect of the furosemide was masked by sodium-conserving responses elicited by the sodium depletion.

**Discussion**

These data demonstrate that furosemide in doses of 0.5 or 2.5 mg/kg stimulates renin release by a mechanism not dependent on changes in total body sodium or water balance. They extend the findings of Meyer et al. (4), who reported that 10 mg furosemide/kg stimulated renin secretion in rabbits despite prevention of fluid depletion by continuous shunting of urine back into the body. Dog and rabbit thus appear to be similar in this respect. Humans also manifest increased renin release in response to furosemide (7, 8), but it is not known whether this stimulation depends on changes in sodium balance. This latter question is of some importance since there are discrepancies between the data reported for ethacrynic acid in man (3) and in the dog (5); the renin-stimulating effect of ethacrynic acid in man did seem to depend in large part on change in plasma volume, whereas dogs manifested an increased renin despite urine shunting which prevented fluid depletion. Another point of great interest is the demonstration by the experiments of Meyer et al. (4) that although the renin-stimulating effect of furosemide was not dependent on volume reduction, it could be abolished by simultaneous volume expansion.

The present experiments also demonstrate that smaller doses of furosemide (0.1 mg/kg) stimulate renin release only when salt depletion is allowed to occur, i.e., when urinary losses are not replaced. Thus, in these low (but definitely natriuretic) doses, the renin response to furosemide is similar to that observed for mercurials and chlorothiazide (2, 9, 10) and, perhaps, ethacrynic acid in humans (3).

What is the stimulus for increased renin release after furosemide without volume depletion? There was no significant change in plasma sodium, and arterial pressure increased in these experiments. The glomerular filtration rate remained constant and renal plasma flow either did not change or increased slightly. Furosemide has been reported to alter the intrarenal blood flow distribution (11), specifically to increase blood flow in the pars radiata of the cortex and to decrease flow in the juxtamedullary cortex and medulla. However, it is difficult to understand how such a change, were it to have occurred in our experiments, would stimulate renin secretion, since most renal renin is located in the outer cortex rather than the juxtamedullary cortex or medulla (12).

We have previously postulated (1) that the sodium load to the macula densa is detected and that there exists an inverse relationship between sodium load and renin release; further, that the link between sodium load and renin release is the rate of sodium transport by the macula densa cells and their intracellular sodium concentration. Thus, an increased delivery of sodium to the macula densa would stimulate sodium uptake by the cells, thereby increasing intracellular sodium concentration, which would, by some mechanism, inhibit renin release. Nash et al. (13) have recently suggested a very similar theory; they hypothesized that the rate of sodium transport across the macula densa into the interstitium is the important factor. Recently, this theory has received strong indirect support from micropuncture studies (14, 15) which demonstrated that, in the loop of Henle, both the rate of sodium reabsorption and the concentration of sodium in the reabsorbate were consistent correlates of flow rate through, and total sodium delivery to, the loop. Just such a relationship would be required for the functioning of the macula densa system hypothesized above (it must be
emphasized, however, that the micropuncture data were for overall loop and early distal function, not for the macula densa itself).

We believe that the effects of furosemide and other natriuretic drugs can all be explained in terms of this macula densa hypothesis. The following observations must be accounted for: (1) Strongly natriuretic doses of furosemide stimulate renin release by a mechanism not dependent on fluid depletion (ref. 4 and the present experiments). (2) Marked expansion of the extracellular or plasma volume blocks this renin-stimulating effect of furosemide (4). (3) Furosemide in small doses (0.1 mg/kg) stimulates renin release only when salt depletion ensues and despite the continued presence of the natriuresis (present experiments). (4) Mercurials and chlorothiazide stimulate renin release only when salt depletion ensues and despite the continued presence of the natriuresis (2, 9, 10). (5) Mercurials and chlorothiazide (as well as acetazolamide) inhibit the renin release induced by reduction of renal perfusion pressure (2, 10, 16). We shall discuss these observations in turn, using as common denominators the assumptions that macula densa sodium transport depends on both the total delivery of sodium to the early distal tubule and the reabsorptive “capacity” of the cells, the latter being inhibited by furosemide and, to a lesser extent, by mercurials and chlorothiazide.

1. Furosemide’s major site of action is the ascending loop of Henle (17-23). Accordingly, the administration of furosemide, without volume depletion, markedly increases the amount of sodium entering the early distal tubule. However, if furosemide also directly inhibited sodium reabsorption (specifically, movement of sodium from lumen into cell) by macula densa cells, then this direct blockade of sodium transport might more than compensate for the reabsorption-stimulating effect of the increased sodium delivery from the loop. The net result would be decreased sodium uptake and enhanced renin release. Unfortunately, there are no direct studies of the effects of furosemide on sodium reabsorption by macula densa cells; however, Rojo-Ortega and his colleagues (personal communication) have demonstrated that furosemide causes a marked acute reduction in G-6PD in macula densa cells as well as the ascending loop of Henle, data which suggest a common site of action. Moreover, data from experiments with frog-skin (24) suggest that furosemide does, indeed, act by reducing the initial entry of sodium into cells.

2. Volume expansion markedly suppresses proximal tubular sodium reabsorption, whereas furosemide exerts only slight if any proximal effect (25). Therefore the combination of volume expansion (which inhibits proximally) and furosemide (which inhibits the loop) should yield a profound increase in flow rate and sodium delivery to the early distal tubule. The reabsorption-stimulating effect of this increased load might overcome the direct furosemide-induced inhibition of transport, and the net result would be normal macula densa sodium transport and no increase in renin release, as reported by Meyer et al. (4).

3. The low dose of furosemide (0.1 mg/kg), without volume depletion, would also fail to stimulate renin secretion if its direct effect on the macula densa at this dose were reduced to a level inadequate to overcome the reabsorption-stimulating effects of the increased sodium load from the loop. On the other hand, when sodium depletion is allowed to occur, then the sodium load leaving the proximal tubule would be decreased (as a result of decreased glomerular filtration rate, increased proximal reabsorption, or both); accordingly, the sodium load to the early distal tubule would increase less than during furosemide without volume depletion and the net result would be decreased cell sodium uptake and increased renin secretion.

4 and 5. The effects of the mercurial diuretics and chlorothiazide (with and without volume depletion) can be analyzed in a similar manner. These natriuretic drugs appear to act, more or less, on various nephron segments (26); certainly their effects on the loop of Henle (and therefore presumably on the macula densa) are less than those of
furosemide (18, 20, 23). In the case of mercurials or chlorothiazide without volume depletion one might expect that the opposed effects of increased sodium load (resulting from proximal or loop inhibition) and direct macula densa inhibition would cancel each other and there would be no change in renin secretion. On the other hand, the superimposition of sodium depletion would reduce the magnitude of this increase in early distal load (as described above) and result in enhanced renin secretion as the direct inhibitory effect predominates. Conversely, when renin secretion is already increased by reduction of perfusion pressure (and, thereby, macula densa sodium load), the load-enhancing effect of the mercurials or chlorothiazide becomes predominant and renin secretion is inhibited. This type of analysis, similar to that presented by Nash et al. for the mercurials (13), adequately explains the paradoxical effects of the mercurials and chlorothiazide in terms of a single macula densa hypothesis and eliminates the need for postulating a second unique type of input to the juxtaglomerular apparatus operating during salt depletion (1, 10).

There is a second, quite different macula densa hypothesis, which was originally postulated by Thurau (27) and is favored by Meyer et al. (4) as an explanation for their furosemide data; namely, that the stimulus for renin release is an increased macula densa intraluminal sodium concentration. However, several aspects of the furosemide data are not consistent with this theory. First, as Meyer et al. (4) have pointed out, it is very unlikely that volume expansion would significantly modify the increased macula densa intraluminal sodium concentration caused by furosemide; yet volume expansion does block furosemide-induced renin secretion. Second, our small, but definitely natriuretic dose of furosemide (0.1 mg/kg) almost certainly raised macula densa intraluminal sodium concentration yet did not induce renin release when volume depletion was prevented. Moreover, in addition to these inconsistencies concerning furosemide, the observations described above for other natriuretic drugs are not explainable in terms of this theory: chlorothiazide and mercurials also increase early distal sodium concentration (18, 20, 23), but do not stimulate renin release when salt depletion is prevented; indeed, they actually inhibit the renin release induced by arterial pressure reduction (2, 10, 16). Finally, at least three other observations are inconsistent with the intraluminal sodium theory: (1) The infusion of either sodium sulfate or hypertonic saline inhibits the renin release induced by arterial pressure reduction (6, 13, 17) despite the fact that the former infusion increases early distal tubular sodium concentration and the latter decreases it (28). (2) Ureteral occlusion during mannitol diuresis stimulates renin secretion (29), although it almost certainly reduces macula densa intraluminal sodium concentration. (3) Reduction of loop flow rate during micropерfusion caused inconsistent changes in early distal sodium concentration (15). In contrast, the sodium load theory can explain all these observations: both sodium sulfate and hypertonic saline increase early distal sodium load (28); ureteral occlusion during mannitol diuresis reduces sodium load (30); reduction of loop flow rate always reduced sodium load (15).

In summary, we believe that the sodium load theory of macula densa function adequately explains the effects of the various natriuretic drugs as well as almost all other circumstances associated with changes in renin secretion (1). Clearly, the operation of such a system during changes in sodium balance (apparently the major determinant of renin secretion) would depend on changes of the glomerular filtration rate and of sodium reabsorption in the proximal tubule and loop of Henle, since these factors ultimately determine sodium load to the macula densa. Finally, although the postulation of a single input to the renin-secreting cells seems justifiable at present, there might very well be other unique inputs. The inhibitory effect of angiotensin on renin secretion is probably such an input (1), and the possibility that the sympathetic nerves, other hormones, and ions other than sodium might act directly on the...
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renin-secreting cells (rather than only indirectly through changes in glomerular filtration rate and sodium reabsorption) remains to be evaluated.

References


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