Inhibition of Furosemide-Induced Increases in Plasma Renin Activity by Amiloride

Kenji Shimoda, M.D., Thomas C. Lee, Ph.D., and Morton H. Maxwell, M.D.

SUMMARY The effect of the potassium-sparing diuretic, amiloride, was studied in conscious rabbits bearing chronic indwelling cannulas to assess whether its reported in vitro kallikrein-inhibiting activity may produce a suppressive effect on furosemide-induced renin secretion similar to that previously demonstrated with another kallikrein inhibitor, aprotinin. Furosemide elicited a rapid and persistent rise in plasma renin activity (PRA), but pretreatment of the same rabbits with a 15-minute intravenous infusion of amiloride, which amounted to 1 mg/kg and commenced at 30 minutes before furosemide, completely prevented this rise. Amiloride also prevented furosemide-induced kaliuresis without an attenuation of the diuretic or natriuretic response and did not alter plasma potassium concentration in the absence of any change in external potassium balance, indicating that suppression of the PRA response is due neither to prevention of extracellular fluid volume contraction nor to the known suppressive effect of hyperkalemia. Mean arterial pressure tended to fall slightly but not significantly with or without amiloride pretreatment. On the basis of these findings and those of our antecedent study with aprotinin, we conclude that the striking similarity between the suppressive effects of two dissimilar inhibitors of kallikrein on pharmacologically evoked renin secretion is consistent with the hypothesis that renal kallikrein participates in the mechanism of renin secretion in vivo. (Hypertension 5: 706-711, 1983)

Key Words • renin secretion • conscious rabbits

Urineary kallikrein of renal origin has been reported to stimulate renin release in vitro directly by superfused rat renal cortical slices, isolated rat glomeruli, and isolated perfused hog kidneys. In our earlier attempt to investigate whether inhibition of kallikrein activity might suppress renin secretion in vivo, we found that both the natriuresis and rise in plasma renin activity (PRA), used as an index of increased active renin secretion, in response to intravenous infusion of aprotinin, a polyvalent bovine proteinase inhibitor that inhibits kallikrein. Since prevention of urinary loss by continuous fluid replacement in our experiment and ureterovenous anastomosis did not prevent the stimulatory effect of furosemide on PRA, we interpreted our results as indicating that the suppression of the furosemide-induced rise in PRA by aprotinin was probably not secondary to suppression of natriuresis. However, because aprotinin also inhibits other serine proteases besides kallikrein, our observations are only consistent with, but do not prove, the possibilities that renal kallikrein may mediate the release of active renin and participate in the natriuretic action of furosemide. As a logical sequel, we have therefore sought to obtain corroborative evidence under similar conditions by using amiloride, an N-amidino pyrazine carboxamide and a potassium-sparing diuretic that Margolius and Chao demonstrated to be an effective inhibitor of renal kallikrein activity in vitro.

Materials and Methods

Male New Zealand white rabbits (2.3 to 3.2 kg) maintained on standard laboratory rabbit chow (Purina) were used. At 2 to 3 days before study, each was anesthetized with sodium thiamylal (30 mg/kg, i.v.) and implanted with one cannula in the left carotid
artery, two cannulas in the left jugular vein, as well as one cannula in the right ureter for subsequent assessments of unilateral renal excretory function as before. All cannulas were exteriorized and anchored with 2-0 silk sutures, the former between the scapulae and the latter on the flank.

Experiments were performed in conscious animals loosely confined in a lucite restraining cage (Plas Laboratories, Lansing, Michigan). Each was studied a second time after an interim of 2 days, once with and once without amiloride pretreatment. When the animal appeared calm and arterial pressure had stabilized, five consecutive urine collections of 30 minutes each were then begun, three during the control period and two after furosemide administration given at time zero. At the beginning of the third control collection period (-30 minutes), either the total dose (1 mg/kg) of amiloride (2 mg/ml in 0.9% NaCl) or an equal volume of vehicle was infused intravenously during the initial 15 minutes of the 30-minute period via a variable speed Harvard infusion pump (Model 944). At the end of this third control period, furosemide (Lasix, Hochst-Roussel Pharmaceuticals, Somerville, New Jersey) was then given as an intravenous bolus injection (0.75 mg/kg) via the second of two jugular cannulas. This dose of the loop diuretic was selected because our previous study showed that, while a lower dose (0.25 mg/kg) produced significant natriuresis, it did not elicit a consistent rise in PRA.

Mean arterial pressure was monitored continuously via the cannula in the carotid artery, using a Statham P23Db pressure transducer connected to a Grass Model 7 polygraph. Specimens for determinations of PRA, plasma sodium and potassium concentrations, as well as excretory rates of urinary volume and electrolytes, were obtained before and at various intervals up to 60 minutes after furosemide administration. Urinary loss was not replaced. PRA was measured by radioimmunoassay, and concentrations of sodium and potassium in plasma and urine by flame photometry.

Because the suppressive effect of aprotinin on furosemide-induced natriuresis (demonstrated in the initial experiment of our antecedent study) was analyzed using a 0.25 mg/kg dose of furosemide, which did not consistently increase PRA, the effect of amiloride on renal excretory function was therefore also investigated in using this lower dose of furosemide in five additional rabbits, lest the higher dose (0.75 mg/kg) used to induce predictable increases in PRA might mask the effect of amiloride on these parameters.

Results were expressed as means ± SEM. Significance of intragroup differences was assessed by Student's paired t test and that between groups, by Student's t test, critical to a 5% level of significance.

Results

Amiloride increased urine volume and sodium excretion during the pretreatment control period before furosemide administration (table 1), but completely prevented the rapid and persistent rise in PRA that occurred in the same rabbits not pretreated with amiloride but given the same high dose (0.75 mg/kg) of the loop diuretic on a different day. Moreover, this phenomenon was not dependent upon any significant change in either the diuretic or natriuretic responses nor their patterns of excretion (table 1). Neither did amiloride affect the magnitudes of these parameters in response to the lower (0.25 mg/kg) dose of furosemide (table 1). In marked contrast to the differences in PRA between groups (figure 1), urine volume and sodium

### Table 1. Renal Excretory Responses to Intravenous Doses of Furosemide in Conscious Rabbits With or Without Prior Amiloride Treatment (1 mg/kg) During Repeated Trials on Separate Days

<table>
<thead>
<tr>
<th></th>
<th>Saline vehicle</th>
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<th>Amiloride</th>
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<tbody>
<tr>
<td></td>
<td>V&lt;sub&gt;u&lt;/sub&gt; (ml/30 min)</td>
<td>U&lt;sub&gt;N&lt;/sub&gt;V (μEq/30 min)</td>
<td>U&lt;sub&gt;P&lt;/sub&gt;V (μEq/30 min)</td>
<td>V&lt;sub&gt;u&lt;/sub&gt; (ml/30 min)</td>
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<tr>
<td>Furosemide (0.75 mg/kg)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>C-1</td>
<td>1.32 ± 0.01</td>
<td>105.1 ± 13.4</td>
<td>73.8 ± 6.7</td>
<td>1.36 ± 0.11</td>
</tr>
<tr>
<td>C-2</td>
<td>1.29 ± 0.06</td>
<td>99.6 ± 19.2</td>
<td>72.0 ± 4.2</td>
<td>1.44 ± 0.09</td>
</tr>
<tr>
<td>C-3</td>
<td>1.23 ± 0.12</td>
<td>102.0 ± 20.4</td>
<td>69.0 ± 6.0</td>
<td>2.67 ± 0.29</td>
</tr>
<tr>
<td>F-1</td>
<td>15.67 ± 1.78*</td>
<td>2001.3 ± 302.4</td>
<td>221.8 ± 34.64</td>
<td>12.73 ± 2.04</td>
</tr>
<tr>
<td>F-2</td>
<td>1.26 ± 0.15</td>
<td>116.3 ± 18.8</td>
<td>73.2 ± 7.0</td>
<td>1.55 ± 0.13</td>
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<tr>
<td>Furosemide (0.25 mg/kg)</td>
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<td></td>
</tr>
<tr>
<td>C-1</td>
<td>1.25 ± 0.09</td>
<td>101.8 ± 12.5</td>
<td>75.4 ± 8.4</td>
<td>1.30 ± 0.06</td>
</tr>
<tr>
<td>C-2</td>
<td>1.31 ± 0.07</td>
<td>103.1 ± 14.5</td>
<td>76.8 ± 7.5</td>
<td>1.36 ± 0.10</td>
</tr>
<tr>
<td>C-3</td>
<td>1.29 ± 0.10</td>
<td>103.3 ± 16.7</td>
<td>73.9 ± 6.2</td>
<td>2.58 ± 0.25</td>
</tr>
<tr>
<td>F-1</td>
<td>9.81 ± 2.21†</td>
<td>978.6 ± 182.4</td>
<td>191.5 ± 28.34</td>
<td>9.45 ± 2.08</td>
</tr>
<tr>
<td>F-2</td>
<td>1.50 ± 0.27</td>
<td>105.0 ± 17.9</td>
<td>99.6 ± 15.4</td>
<td>1.51 ± 0.24</td>
</tr>
</tbody>
</table>

V<sub>u</sub> = urinary volume; U<sub>N</sub>V = sodium excretion; U<sub>P</sub>V = potassium excretion; C = control; F = furosemide.

Significance of differences compared to periods C-1 or C-2 in respective trials: *p < 0.05; †p < 0.025; ‡p < 0.005. Amiloride or vehicle given in C-3.
excretion in all groups peaked at 5 to 10 minutes and returned to control levels at 30 to 45 minutes after either dose of furosemide. Because statistical comparisons between the renal excretory responses to the high dose of furosemide in the presence or absence of amiloride and either of the values obtained during the immediately preceding control period (amiloride or vehicle infusion) or the initial two 30-minute control periods were not sufficiently different to possibly obscure the demonstration of amiloride's effect on furosemide-induced changes, the data obtained for the first two control periods as well as the two periods after furosemide were combined and are graphically depicted for comparison in figures 2–4 as rates of excretion per hour for urine, sodium, and potassium outputs, respectively.

No discernible changes in plasma sodium concentration were seen at 60 minutes after administration of furosemide, either when given alone or in combination with amiloride. Similarly, the plasma potassium concentrations of both groups also did not change noticeably from their respective control values, nor did any value of one group differ from any value of the other group, despite the fact that amiloride was antikaliuretic during the 30-minute pretreatment period in which urinary potassium excretion fell from 72.7 ± 6.6 to 53.9 ± 4.7 μEq/30 min (p < 0.025) and completely prevented furosemide-induced kaliuresis (fig. 4). In the

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**Figure 1.** Suppression of the furosemide-induced increase in plasma renin activity in conscious rabbits by infusion of amiloride (1 mg/kg) given at 30 minutes before furosemide.

**Figure 2.** Diuretic responses to furosemide (F = 0.75 mg/kg) given alone and in combination with amiloride (A = 1 mg/kg). C = control values before drug administration during repeated trials in the same rabbits. See text for specifics.

**Figure 3.** Natriuretic responses to furosemide given alone and in combination with amiloride. Abbreviations and dosages are as in figure 2.
Discussion

Prevention of urinary losses either by ureteral-venous anastomosis or by parenteral replacements of fluid and sodium in our previous study did not prevent the stimulated rise in PRA in response to furosemide administration. The inhibition of this rise in PRA by amiloride without any significant attenuation of the natriuretic effect of furosemide in the present experiment further strengthens our previous suggestion that the suppression by aprotinin of the furosemide-induced rise in PRA was probably not due to a concomitant suppression of furosemide-induced natriuresis but to an inhibition of renal kallikrein activity. Urinary kallikrein excretion, the commonly used but presumptive index of renal kallikrein activity, is known to be stimulated by furosemide and its increase has been reported to parallel the increase in PRA. Moreover, patients with essential hypertension and low basal rates of kallikrein excretion have been reported to exhibit blunted increases in PRA associated with concomitantly reduced increments in urinary kallikrein excretion when compared to normal subjects in response to furosemide. In addition, besides our demonstration of its suppressive effect on furosemide-stimulated renin release in the rabbit, aprotinin was subsequently shown to inhibit both of these effects of furosemide simultaneously in the rat.

Although components of the kallikrein-kinin system were not measured in our studies, the fact that aprotinin and amiloride both inhibit glandular kallikrein activity strongly suggests that their strikingly similar ability to suppress the stimulated rise in PRA produced by furosemide might be mediated via an inhibition of this system. Demonstrations that aprotinin and amiloride abolished the stimulatory effect of urinary kallikrein on renin release in vitro support this possibility. However, it may be reasonable to question whether the increase in urinary kallikrein excretion stimulated by furosemide is pharmacologically significant or only coincidental with other effects of this loop diuretic, since different classes of diuretics show a similar effect. Based on the observations that their hypotensive efficacy correlated with alterations in urinary kallikrein excretion, it was recently proposed that the increases stimulated by furosemide could indeed be pharmacologically significant.

Unlike its similarity of effect as aprotinin in suppressing furosemide-induced renin release, amiloride did not affect the diuretic or natriuretic response to either the high or low dose of furosemide despite its intrinsic, but mild, natriuretic property which was also evident in the present study during the pretreatment period before furosemide. It is possible that the antinatriuretic effect of aprotinin is due to its inhibition of a serine protease other than kallikrein and that amiloride, whose mechanism of action as a sodium channel blocker in diverse epithelia is unresolved, may possess an independent natriuretic action unrelated to its kallikrein-inhibiting activity. Nevertheless, the two agents’ dissimilarity of effects on the renal excretory responses to furosemide raises the question as to whether their shared ability to inhibit kallikrein activity underlies a common mechanism by which both can suppress furosemide-induced renin release. Conversely, the natriuretic action of furosemide and its activation of the processes regulating renin release may also be mediated via different mechanisms. It is pertinent to note that, somewhat analogous to the observations in this and our previous study, Wilson et al. recently showed in pentobarbital-anesthetized dogs that an intravenous dose (1 mg/kg) of furosemide greater than that (0.1 mg/kg) which caused near maximal diuresis and natriuresis was required to increase PRA and 6-keto-prostaglandin F excretion. The latter is a stable metabolite of renal prostacyclin (PGI,) and its active stable metabolite (6-keto-PGE,) which, like PGE, are potent stimulators of renin secretion in vitro and in vivo and whose synthesis can be stimulated by activation of the renal KKS as well as inhibited by aprotinin.

Furosemide-induced changes in PRA are renal-dependent, and in the rabbit it has been shown that the proportion of plasma active renin concentration increased during furosemide administration with replacement of volume loss. Several factors are consis-
tent with the possibility that the suppressive effects of aprotinin\textsuperscript{5, 12} and amiloride on the pharmacologically evoked rise in PRA may be secondary to an inhibition of intrarenal kallikrein activity. These factors include demonstrations that kallikrein administration can increase active renin while decreasing inactive renin concentration in the effluent of isolated perfused hog kidneys,\textsuperscript{4} which has been reported to release both forms in parallel under a variety of conditions including furosemide administration,\textsuperscript{24} and that the human kidney can activate circulating inactive renin.\textsuperscript{22} It has also been reported that aprotinin reduced plasma active renin concentration without a change in plasma inactive renin concentration in patients with essential hypertension.\textsuperscript{26} Plasma concentrations of inactive renin (prorenin) were not measured in our studies. Hence, although our data support the hypothesis that kallikrein may be a physiological activator of prorenin,\textsuperscript{27} they do not distinguish whether it was activation or release of renin that was suppressed, since urinary kallikrein and kinins were recently reported to have independent stimulatory effects on renin release by isolated renal glomeruli in vitro.\textsuperscript{3}

Surreptitious ingestions of furosemide are known to induce pseudo-Bartter’s syndrome,\textsuperscript{23} and amiloride treatment was recently reported to decrease PRA significantly from 25.3 ± 1.9 to 11.9 ± 0.4 ng Al/mL/hr in five patients with Bartter’s syndrome,\textsuperscript{29} a condition characterized in part by a defect in chloride transport in the thick ascending limb of the loop of Henle\textsuperscript{30} and by very high plasma levels of active renin, with an essential absence of plasma inactive renin\textsuperscript{31} as well as markedly increased rates of urinary kallikrein excretion and hyperbradykininemia.\textsuperscript{32, 33} Since amiloride is antikaliuretic and an increase in plasma potassium concentration is known to inhibit renin secretion,\textsuperscript{31} an increase in plasma potassium concentration could conceivably contribute to or be responsible for the suppressive effect of amiloride on renin secretion. However, plasma potassium concentration did not change during amiloride treatment in our acute experiment despite the significant antikaliuresis observed before and after furosemide administration. Thus, in the absence of any change in the external balance of potassium, the potential suppressive effect of this ion on renin secretion was not likely to have contributed significantly to amiloride’s effect, particularly since large amounts of infused potassium sufficient to significantly elevate plasma potassium concentration was found to be necessary before the effect is demonstrable.\textsuperscript{34}

Thus, together with the demonstration of amiloride’s effect in patients with Bartter’s syndrome,\textsuperscript{29} the results of the present study suggest that, apart from its proven antikaliuretic efficacy,\textsuperscript{13, 16, 29} the inhibition of renin activation and/or release by amiloride could potentially have therapeutic significance in countering reactive hyperreninemia which contributes to limiting the therapeutic efficacy of diuretics\textsuperscript{35} and for reducing hyperangiotensinemia in renin-dependent states of hypertension. Although the usual daily doses of amiloride (5 to 20 mg) used in conjunction with other diuretics to prevent diuretic-induced kaliuresis had not been shown to suppress PRA and, in fact, may at times contribute to slight increases due to the greater natriuretic potency of the combination,\textsuperscript{15, 16} it does not preclude the possibility that higher doses may suppress renin secretion if untoward side effects (e.g., hyperkalemia) can be avoided during prolonged use. This possibility deserves investigation.

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References

Inhibition of furosemide-induced increases in plasma renin activity by amiloride.
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