Control of Renin Secretion in the Dog

EFFECTS OF FUROSEMIDE ON THE VASCULAR AND MACULA DENS A RECEPTORS

By William A. Corsini, Jerry B. Hook, and Michael D. Bailie

ABSTRACT

Experiments were undertaken to investigate further the effect of furosemide on renin secretion in the anesthetized dog. To separate the effects of the macula densa and the baroreceptor mechanisms, experiments were conducted in kidneys made nonfiltering by combining 2.5 hours of renal ischemia with ureteral ligation. Furosemide, in a dose of 5 mg/kg, increased renin secretion and decreased renal resistance in dogs with a nonfiltering kidney. Prior dilation of the nonfiltering kidney with either acetylcholine or papaverine prevented changes in both resistance and renin secretion. However, following dilation of the intact filtering kidney with acetylcholine, furosemide caused an increase in renin secretion. Infusion of d,l-propranolol decreased renin secretion in both the filtering and the nonfiltering kidneys. Following propranolol treatment, furosemide increased renin secretion in the filtering kidney but had no effect on renal resistance. These experiments indicate that furosemide stimulates renin secretion by both the macula densa and the baroreceptor mechanisms. The data suggest that stimulation of the sympathetic nervous system may alter renin secretion by modulating the renal baroreceptor, but sympathetic innervation does not appear to be involved in the macula densa mechanism.

The diuretic, furosemide, stimulates renin secretion by a mechanism independent of a decrease in extracellular fluid volume (1-3). This effect has been explained in terms of the action of the drug on sodium transport at the macula densa portion of the distal tubule: inhibition of sodium transport at the macula densa causes a decrease in the interstitial sodium concentration which leads to an increase in renin secretion (1, 4).

Recently, Blaine et al. (5) have reported the results of experiments with a nonfiltering kidney model in which sodium is prevented from reaching the macula densa. Plasma renin activity increases in this nonfiltering kidney following suprarenal aortic constriction or hemorrhage (5), and the effect is independent of sympathetic innervation (6, 7). These findings provide evidence for a renal baroreceptor mechanism for control of renin secretion that acts independently of the macula densa or the sympathetic nervous system.

Furosemide inhibits electrolyte transport in the ascending limb of the loop of Henle and also causes renal vasodilation leading to an increase in renal blood flow (1-3). Therefore, the diuretic may stimulate renin secretion through two separate mechanisms involving either vasodilation of the afferent glomerular arteriole or modification of sodium (chloride) transport at the macula densa portion of the distal tubule. To define further the mechanisms by which furosemide increases renin secretion, experiments were carried out in dogs utilizing the nonfiltering kidney model. The results of these studies demonstrate that furosemide has an effect on two mechanisms which function independently.

Methods

Animal Preparation.—Experiments were carried out on male, mongrel dogs weighing 9-18 kg. Preparation of a nonfiltering kidney was undertaken using the method of Blaine et al. (5). Anesthesia was induced with sodium thiopental (18 mg/kg, iv) and maintained with methoxyflurane. The kidney was exposed through a retroperitoneal flank incision using sterile technique. Renal ischemia was produced by occluding the renal artery for 2.5 hours. Just prior to occlusion of the renal artery, the ureter was ligated approximately 5 cm from the renal pelvis. After the ischemic period, the arterial clamp was removed, the incision was closed, and the dog was allowed to recover. Some experiments were performed on dogs with a single nonfiltering kidney. In these dogs, the contralateral normal kidney was removed through a midline incision 3-4 days after the initial procedure. Dogs with a single normal kidney acted as controls for this group. The unilateral nephrectomy was performed 24 hours prior to the experiment. In a second group of dogs, the contralateral normal kidney was left in place and acted as the control.

On the morning of the experiment, the dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) following insertion of a cuffed endotracheal tube, all of the dogs were artificially ventilated (Harvard Apparatus).
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Co., Inc.). A polyethylene catheter was inserted through the left femoral artery into the abdominal aorta for the collection of arterial blood samples and the recording of arterial blood pressure with a strain-gauge pressure transducer (Statham P23Dd) and a direct-writing oscillograph (Grass Polygraph). A polyethylene catheter was also inserted into the femoral vein for injection of furosemide and infusions of dl-propranolol and maintenance doses of anesthetic. In dogs with both a filtering and a nonfiltering kidney, a midline abdominal incision was used. After exposure of the vessels, a curved 18-gauge needle attached to a polyethylene catheter was inserted directly into the renal vein for collection of renal venous blood samples. A noncannulating electromagnetic flowmeter probe (Carolina Medical Electronics, Inc.) was placed on the renal artery, and renal blood flow was recorded on the oscillograph. In dogs with two kidneys, a needle was placed in each renal vein and a flow probe was placed around each renal artery to simultaneously collect blood samples and record renal blood flow from each organ. In some experiments, a curved 22-gauge needle attached to a polyethylene catheter was placed directly into the renal artery for the infusion of saline, acetylcholine, or papaverine. A minimum of 1 hour was allowed for the dogs to recover from surgery before the experiment was started. At the end of each experiment in which a nonfiltering kidney was used, the kidney was examined for nonfiltration by observing the appearance of lissamine green dye injected into the renal artery as described by Blaine et al. (5). In addition, the ligated ureter was cannulated proximal to the ligation, and two or three 10-minute inulin clearances were obtained. Kidneys without dye in the surface tubules and with an inulin clearance of less than 2 ml/min were accepted as nonfiltering. In dogs with intact kidneys, the ureter was cannulated prior to the start of the experiment, and urinary fluid losses were continually replaced by an infusion of isotonic saline into the femoral vein.

Experiment A: Effects of Furosemide in Dogs with a Single Nonfiltering Kidney.—In seven dogs, after obtaining three control samples of arterial and renal venous blood, furosemide (5 mg/kg) was injected into the femoral vein. Additional blood samples were then obtained 5, 10, 20, and 30 minutes after the furosemide injection.

Experiment B: Effects of Furosemide after Renal Vasodilatation in Dogs with a Single Nonfiltering Kidney.—In 14 dogs, saline was infused continuously (0.2–0.8 ml/min) into the renal artery during the 1-hour recovery period following surgery. Three control samples of arterial and renal venous blood were then obtained. The renal arterial infusion solution was then changed to one containing either papaverine or acetylcholine. Both drugs were infused at a rate which increased renal blood flow maximally without greatly reducing systemic blood pressure. The doses of papaverine and acetylcholine necessary for vasodilatation were 2-8 mg/min and 4 μg/kg min−1, respectively. After allowing 20 minutes for stabilization of renal blood flow, three blood samples were drawn 5 minutes apart. While the renal arterial infusion of papaverine or acetylcholine was continuing, furosemide (5 mg/kg) was injected intravenously and blood samples were drawn 5, 10, 20, and 30 minutes after the injection.

Experiment C: Effects of Furosemide after Renal Vasodilatation in Dogs with a Single Filtering Kidney.—In seven dogs, saline was infused into the renal artery (0.2–0.8 ml/min) during the 1-hour recovery period following surgery and during the collection of three control blood samples. Following the collection of control samples, acetylcholine was infused into the renal artery of the dogs at a rate necessary to achieve maximum renal vasodilatation. Blood samples for the determination of renin secretion before and after the furosemide injection were obtained as described in the preceding protocol for experiment B.

Experiment D: Effects of Propranolol and Furosemide in Dogs with Both a Filtering and a Nonfiltering Kidney.—The fourth experimental protocol was carried out in eight dogs with both a filtering and a nonfiltering kidney. Two control arterial and renal blood samples were obtained at 10-minute intervals. After obtaining the control blood samples, the beta-adrenergic antagonist, dl-propranolol was given intravenously as a bolus (1 mg/kg) followed by a continuous infusion into the femoral vein at a rate of 1 mg/kg hour−1. After allowing 20 minutes for the establishment of equilibrium, two arterial and renal venous blood samples were obtained at 10-minute intervals. Furosemide (5 mg/kg) was then injected into the femoral vein, and two more blood samples were obtained.

Analytical and Statistical Methods.—Hematocrit was determined on all arterial blood samples by the micro-method. Renin activity in femoral arterial and renal venous blood samples was determined by the method of Haber et al. (8) using a renin activity radioimmunoassay kit for angiotensin I (Schwarz-Mann). Arterial and renal venous plasma and urine samples were analyzed for inulin concentration using the diphenylamine method of Walser et al. (9), or samples containing 14C-labeled inulin were counted in a liquid scintillation counter.

Renin secretion was calculated as the product of the renal plasma flow and the renal venous-arterial renin activity difference and was expressed as nanograms secreted per minute. Blood pressure and renal blood flow were obtained directly from the recordings, and renal plasma flow was calculated from the blood flow and the hematocrit.

Since there was significant heterogeneity of variance in renin secretion, the data were plotted on semilogarithmic graphs, and statistical analyses were performed on the logarithmic transformation. The data on renin secretion and hemodynamics were analyzed using a two-way analysis of variance (10). The 0.05 level of probability was used as the criterion of significance.

Results

Experiment A: Effects of Furosemide in Dogs with a Single Nonfiltering Kidney.—In the seven dogs in this group, there was a significant threefold increase in renin secretion following the administration of furosemide (Fig. 1). This change in renin...
secretion was associated with a decrease in renal resistance, an increase in renal blood flow, and no change in systemic blood pressure (Table 1).

Experiment B: Effects of Furosemide after Renal Vasodilation in Dogs with a Single Nonfiltering Kidney.—Both acetylcholine and papaverine produced a fall in renal resistance (Table 1). Furosemide caused no further decrease in resistance in the dogs treated with acetylcholine (Table 1), although it did depress renal resistance further in the dogs receiving papaverine (Table 1). When furosemide was administered following the infusion of acetylcholine or papaverine into the renal artery, renin secretion did not increase (Fig. 2).

Experiment C: Effects of Furosemide after Renal Vasodilation in Dogs with a Single Filtering Kidney.—Renin secretion after renal vasodilation with acetylcholine was highly variable, ranging from 200 to 5,000 ng/min. Nevertheless, in dogs with a single filtering kidney, furosemide produced significant increases in renin secretion (Fig. 3). The effect of furosemide in these dogs was all the more striking because in two of the seven animals renin secretion before furosemide administration was very high at approximately 3,000 ng/min and did not rise further following the furosemide injection. There was no further change in renal resistance when furosemide was given to these dogs (Table 2).

Experiment D: Effects of Propranolol and Furosemide in Dogs with Both a Filtering and a Nonfiltering Kidney.—In five dogs, the administration of propranolol suppressed renin secretion in both the filtering and the nonfiltering kidney (Fig. 4). Renin secretion during control periods was less in the nonfiltering kidney. Furosemide significantly increased renin secretion in the filtering kidney but did not change renin secretion in the nonfiltering kidney.

In three additional dogs, renin secretion was not suppressed by propranolol, and therefore the data were excluded from Figure 4. However, as in Figure 4, furosemide increased renin secretion in the filtering kidney only. Since propranolol produced a proportional fall in systemic blood pressure and renal blood flow in both kidneys, there was no change in renal resistance (Table 3). Changes in renal hemodynamics after furosemide administration were similar in both kidneys.

Discussion

The secretion of renin appears to be controlled from at least two sites within the kidney: the

### TABLE 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>N</th>
<th>BP (mm Hg)</th>
<th>RBF (ml/min)</th>
<th>RR (pru)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>103</td>
<td>76.2</td>
<td>2.0</td>
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<tr>
<td>Furosemide</td>
<td>99</td>
<td>92.0</td>
<td>-0.4 ± 0.12*</td>
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<tr>
<td>Control</td>
<td>7</td>
<td>109</td>
<td>74.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>106</td>
<td>139</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>103</td>
<td>129</td>
<td>-0.8 ± 0.18*</td>
<td>1.1</td>
</tr>
<tr>
<td>Change ± se</td>
<td>-2 ± 2.9</td>
<td>64 ± 24*</td>
<td>9.6 ± 3.8*</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>128</td>
<td>116</td>
<td>1.4</td>
</tr>
<tr>
<td>Papaverine</td>
<td>108</td>
<td>145</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Change ± se</td>
<td>-19.8 ± 4.4*</td>
<td>30 ± 15</td>
<td>-0.57 ± 0.16*</td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>93</td>
<td>145</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Change ± se</td>
<td>-14.6 ± 2.3*</td>
<td>0.6 ± 7.7</td>
<td>-0.1 ± 0.03*</td>
<td></td>
</tr>
</tbody>
</table>

BP = mean systemic blood pressure, RBF = renal blood flow, RR = renal resistance, and pru = peripheral resistance units. * P < 0.05.
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Top: Effect of furosemide (F) on renin secretion following vasodilation with acetylcholine (A) in dogs with a single nonfiltering kidney. Means ± se are shown. A indicates renin secretion during acetylcholine administration alone; F indicates renin secretion following furosemide administration during infusion of acetylcholine into the renal artery. Bottom: Same as the top section except that papaverine (P) is being infused into the renal artery.

Tubular and vascular sides of the juxtaglomerular apparatus. Tubular control of renin secretion is initiated at the macula densa portion of the distal tubule and has been described by several authors as the macula densa mechanism (3, 4, 11). Vascular control of renin secretion is thought to be initiated in the afferent glomerular arteriole. One mechanism of control of renin secretion at this site, the baroreceptor mechanism, has been postulated to be sensitive to factors including blood pressure, the vascular transmural pressure gradient, and the wall tension of the afferent arteriole (5, 12, 13). A second factor in the control of renin secretion on the vascular side of the juxtaglomerular apparatus, the sympathetic nervous system, may exert an effect by three mechanisms: (1) direct action on the juxtaglomerular cells, since the area of the juxtaglomerular cells has been shown to be innervated by sympathetic fibers (14), (2) modulation of the renal baroreceptor via alterations in the tone of smooth muscle in the afferent arteriole (15), and (3) alteration of sodium delivery to the macula.

FIGURE 2

EFFECT OF FUROSEMIDE ON RENIN SECRETION following vasodilation with acetylcholine (A) in seven dogs with a single filtering kidney. Means ± se are shown. A indicates renin secretion during acetylcholine administration alone; F indicates renin secretion following furosemide administration during infusion of acetylcholine into the renal artery. Furosemide produced a significant increase in renin secretion (P < 0.05).

TABLE 2

Effects of Furosemide on Renal Hemodynamics following Vasodilation with Acetylcholine in Dogs with a Single Filtering Kidney

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>BP (mm Hg)</th>
<th>RBF (ml/min)</th>
<th>RR (pru)</th>
</tr>
</thead>
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<tr>
<td>Control</td>
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<td>117</td>
<td>141</td>
<td>0.98</td>
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<tr>
<td>Acetylcholine</td>
<td>114</td>
<td>186</td>
<td>0.74</td>
<td></td>
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<tr>
<td>Change ± se</td>
<td>-3 ± 3</td>
<td>45 ± 13.2*</td>
<td>0.23 ± 0.05*</td>
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</tr>
<tr>
<td>Furosemide</td>
<td>112</td>
<td>176</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Change ± se</td>
<td>-2 ± 3</td>
<td>-10 ± 8.4</td>
<td>0.02 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations are the same as they are in Table 1.
* P < 0.05.
The precise extent to which each of these mechanisms is involved in the control of renin secretion has not been assessed, since it is difficult to isolate a single mechanism in experiments designed to alter renin secretion. For example, in experiments in which hemorrhage is used as a stimulus, reduced blood pressure may stimulate the baroreceptor, reduced glomerular filtration may stimulate the macula densa receptor, or the decreased intravascular volume may stimulate the sympathetic nervous system, all three leading to an increase in renin secretion. Thus, under these conditions, renin secretion following hemorrhage can only be explained in terms of all three mechanisms.

Recently, the tubular and vascular effects on renin secretion have been differentiated by the use of the nonfiltering kidney model (5). The usefulness of the model is derived from the blockade of sodium delivery to the macula densa portion of the distal tubule and the resultant inactivity of the macula densa mechanism. Therefore, results of experiments using this model can be explained in terms of the vascular site of control only.

To demonstrate the blockade of glomerular filtration, Blaine and coworkers (5) looked for lissamine green dye in the surface tubules following injection of the dye into the renal artery. Like these authors, we were unable to observe the appearance of the dye in the surface tubules. In addition, we found that the clearance of inulin was less than 2 ml/min when the ureter of the nonfiltering kidney was cannulated proximal to the ligature. The glomerular filtration rate prior to cannulation should have been less than this value.

Furosemide, a very potent diuretic, has been previously reported to increase renin secretion by blocking sodium (chloride) transport at the macula densa site (1-3). The diuretic has also been shown to decrease renal resistance independent of volume depletion (1-3). Since reductions in renal resistance following hemorrhage, suprarenal aortic constriction, or the infusion of various drugs increase

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**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>Filtering kidney</th>
<th>Nonfiltering kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BP (mm Hg)</td>
<td>RBF (ml/min)</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>112</td>
</tr>
<tr>
<td>Propranolol</td>
<td>103</td>
<td>139</td>
</tr>
<tr>
<td>Change ± SE</td>
<td>-8.9 ± 2.9</td>
<td>17.8 ± 7.2</td>
</tr>
<tr>
<td>Furosemide</td>
<td></td>
<td>98</td>
</tr>
<tr>
<td>Change ± SE</td>
<td>-4.6 ± 2.3</td>
<td>-13 ± 6.3</td>
</tr>
</tbody>
</table>

Abbreviations are the same as they are in Table 1.

*P < 0.05.
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experiments, furosemide produced a concomitant increase in renin secretion and decrease in renal resistance in dogs with a nonfiltering kidney (Fig. 1, Table 1). These findings demonstrate that furosemide increases renin secretion despite the absence of delivery of fluids to the macula densa portion of the distal tubule. Under these conditions, the rise in renin secretion must result from an effect at the vascular side of the juxtaglomerular apparatus. The decrease in renal resistance resulting from injection of furosemide is consistent with an effect on the baroreceptor. However, a possible action on the sympathetic nervous system cannot be excluded.

If furosemide exerts an effect on renin secretion through the baroreceptor mechanism, then this response should be blocked by vasodilation of the renal vasculature prior to furosemide injection. In the present experiments, the nonfiltering kidney was vasodilated with the smooth muscle paralyzing agent, papaverine, or the parasympathomimetic drug, acetylcholine. Furosemide failed to increase renin secretion or decrease renal resistance in the nonfiltering kidney diluted with acetylcholine (Table 1, Fig. 2). Although furosemide reduced renal resistance in the nonfiltering kidney vasodilated with papaverine, renin secretion was not altered. (Table 1, Fig. 2).

In these latter experiments, the fall in renal resistance following furosemide administration probably resulted from incomplete renal vasodilation from a suboptimal dose of papaverine. However, the dose of papaverine could not be increased further because of the fall in systemic blood pressure which followed larger doses.

These data demonstrate that furosemide can stimulate renin secretion from the vascular side of the juxtaglomerular apparatus. Furthermore, the data suggest that the primary mechanism of renin control at the vascular site is the baroreceptor. The results do not exclude a role for the sympathetic nervous system, but if the nervous system is involved it modulates the baroreceptor instead of stimulating the juxtaglomerular apparatus directly. The possibility also exists that papaverine and acetylcholine may block renin secretion by direct action on the juxtaglomerular cells.

To assess this possibility, acetylcholine was administered to dogs with a filtering kidney. Papaverine was not administered as a vasodilating drug in the filtering kidney because of the fall in systemic blood pressure produced at even minimum vasodilating doses. In dogs with a single filtering kidney dilated with acetylcholine, furosemide produced a statistically significant rise in renin secretion. This increase occurred in spite of the fact that two dogs had control rates of renin secretion greater than 3,000 ng/ml and did not demonstrate an increase in renin secretion following furosemide administration. This experiment suggests that acetylcholine does not directly inhibit the juxtaglomerular cells and, furthermore, demonstrates the independent effect of furosemide on the macula densa mechanism.

d,/-Propranolol has been reported to suppress renin secretion, possibly by an effect on beta receptors at sympathetic nervous terminals in the kidney. The drug is also known to have membrandistabilizing properties similar to those of local anesthetics (17) which could affect the baroreceptor. In the present experiments, propranolol produced a fall in renin secretion in both the filtering and the nonfiltering kidney in five of eight dogs (Fig. 4). In those dogs in which propranolol suppressed renin secretion, a proportionately greater decrease was observed in the nonfiltering kidney, suggesting that propranolol blocks renin secretion at the vascular site. The administration of furosemide to all eight dogs increased renin secretion in the filtering kidney but not in the nonfiltering kidney; renal resistance was unaltered by propranolol or furosemide in either kidney.

This result also suggests that the beta-adrenergic blockade modifies the vascular receptor but has less effect on the macula densa receptor. It should also be kept in mind that the membrane-stabilizing properties of propranolol could explain the blockade of renin secretion following furosemide administration, since the decrease in renal resistance usually seen after furosemide is given did not occur. The present experimental design does not allow us to separate the two effects of propranolol.

The results of experiments reported in this paper support the concept that two separate sites are involved in the control of renin secretion. The vascular site located in the afferent glomerular arteriole is sensitive to changes in vasomotor tone and appears to be modulated by the sympathetic nervous system. The tubular site is sensitive to changes in delivery of sodium to the macula densa or in the sodium concentration at that site and appears to be independent of the sympathetic nervous system. The vasomotor tone at the vascular site may also modify the response at the tubular site by changing the glomerular filtration rate and secondarily the delivery of sodium to the distal tubule. It is unclear whether the sympathetic

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nervous system represents a third independent control mechanism or primarily modulates the response at the vascular site. The present experiments are compatible with but do not prove the latter hypothesis.

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