**Control of Renin Release**

**Effects of \(<\)-Propranolol and Renal Denervation on Furosemide-Induced Renin Release in the Dog**

JEFFREY L. OSBORN, JERRY B. HOOK, AND MICHAEL D. BAILIE

**SUMMARY** We determined the effects of \(<\)-propranolol and renal denervation on furosemide-induced renin release in the anesthetized dog. \(\alpha\)-Propranolol possesses only membrane-stabilizing properties, whereas the \(\beta\)-isomer produces beta adrenergic blockade. To separate the vascular and macula densa mechanisms of the juxtaglomerular apparatus effectively, nonfiltering kidneys were produced by combining 2.5 hours of renal ischemia with ureteral ligation. In some dogs, renal denervation was accomplished by relocating the nonfiltering kidney into the neck during the 2.5-hour ischemic interval. Administration of \(\alpha\)-propranolol in a priming dose of 1 mg/kg, iv, followed by an intravenous infusion of 1 mg/kg per hour decreased renin release in both the filtering and nonfiltering kidney. Subsequent furosemide injection (5 mg/kg, iv) failed to increase renin release in the nonfiltering kidney. Similarly, after the infusion of lidocaine into the renal artery of the nonfiltering kidney (1 mg/kg per hour), furosemide did not alter renin release. In the denervated nonfiltering kidney, furosemide in a dose of 5 mg/kg, iv, increased renin release and decreased renal resistance. Treatment with \(\alpha\)- or \(\beta\)-propranolol decreased renin release in five out of six denervated nonfiltering kidneys. Following propranolol, furosemide failed to increase renin release. These results demonstrate that the ability of \(<\)-propranolol to decrease renin release may be due partially to the membrane-stabilizing activity of the \(\beta\)-isomer. Stimulation of renin release by furosemide occurs at both the vascular and macula densa sites which may act independently in the control of renin release. The data demonstrate that, whereas renal sympathetic innervation may modulate renin release under a variety of circumstances, this innervation is not an absolute requirement for renin release at the juxtaglomerular apparatus.

**Methods**

Experiments were conducted on male, mongrel dogs weighing 9-25 kg. The nonfiltering kidney was prepared utilizing the nonfiltering kidney model developed by Blaine et al.\(^\text{12}\) The effects of \(d,l\)-propranolol, \(r\)-propranolol, lidocaine, and renal denervation on furosemide-induced renin release were determined.

The macula densa mechanism located in the distal tubule,\(^\text{1}\) the vascular (baroreceptor) mechanism in the afferent glomerular arteriole,\(^\text{2}\) and the sympathetic innervation of the juxtaglomerular cells\(^\text{3-4}\) are involved in the regulation of renin release from the kidney. Recently, stimulation of renin release by the diuretic furosemide was shown to occur through both the macula densa and baroreceptor mechanisms.\(^\text{5}\) The macula densa receptor is responsive to alterations in sodium or possibly chloride transport.\(^\text{6-8}\) Furosemide, which blocks the transport of sodium (chloride) in the ascending limb, may alter renin release at the macula densa receptor by this mechanism. The vascular receptor may be stimulated by changes in afferent arteriolar wall tension\(^\text{9}\) or changes in the vascular transmural pressure gradient.\(^\text{10}\) Furosemide stimulates renin release on the vascular side of the juxtaglomerular apparatus (JGA) by vasodilatation.\(^\text{7}\)

There are at least two mechanisms by which the sympathetic nerves may regulate renin release. First, changes in catecholamine release at the juxtaglomerular apparatus\(^\text{6}\) may modify renin release directly. Second, renin release may be altered through modulation of the vascular receptor by the sympathetic nervous system. To define further the role of the vascular receptor and renal innervation in the control of renin release, experiments were conducted utilizing the nonfiltering kidney model developed by Blaine et al.\(^\text{12}\)
and for administration of maintenance doses of anesthetic. In all experiments, renal blood flow was measured by placing noncannulating electromagnetic flowmeter probes on the renal arteries (Carolina Medical Electronics) and recording flow simultaneously on the oscillograph. Renal venous blood samples were collected by placing a curved 20-gauge needle attached to polyethylene tubing into the renal vein. At the end of each experiment, a polyethylene catheter was placed in the ureter of the nonfiltering kidney and the clearance of inulin was observed in two clearance periods of 10 minutes each. If the glomerular filtration rate was greater than 3 ml/min, the experiment was rejected.

EXPERIMENT A: EFFECT OF d-PREPRANOLOL ON FUROSEMIDE-INDUCED RENIN RELEASE IN THE FILTERING AND NONFILTERING KIDNEY

In six dogs, both the filtering and nonfiltering kidney were exposed via a midline incision. Flowmeter probes were placed on each renal artery and renal blood flow was measured simultaneously. A 1-hour recovery period was allowed following surgery on each animal. After recovery, two control arterial and renal venous samples were obtained from each kidney at 10-minute intervals. d-Prepranolol (1 mg/kg, iv) then was injected in a bolus followed by a continuous infusion of 1 mg/kg per hour. Twenty-five minutes were allowed for equilibration, and two renal venous and arterial samples again were collected from the filtering and nonfiltering kidney at 10-minute intervals. Furosemide (5 mg/kg, iv) then was injected. Following a 10-minute equilibration period, two blood samples were obtained at 10-minute intervals.

EXPERIMENT B: EFFECT OF LIDOCAINE ON FUROSEMIDE-INDUCED RENIN RELEASE IN THE NONFILTERING KIDNEY

In six dogs, the effect of lidocaine on furosemide-induced renin release was determined. The retroperitoneal flank incision was reopened and only the nonfiltering kidney exposed. The renal artery and vein were then isolated. A curved 22-gauge needle attached to polyethylene tubing was placed in the renal artery distal to the flow probe for the intrarenal infusion of lidocaine. After the collection of two control arterial and renal venous blood samples, lidocaine was infused intrarenally for 25 minutes at a rate of 2 mg/kg per hour, and then the infusion rate was reduced to 1 mg/kg per hour. Two blood samples then were collected at 10-minute intervals. Furosemide (5 mg/kg, iv) then was injected, and again, following a 10-minute equilibration period, blood samples were obtained.

EXPERIMENT C: EFFECT OF RENAL DENERVATION ON FUROSEMIDE-INDUCED RENIN RELEASE IN THE NONFILTERING KIDNEY

Denervated, nonfiltering kidneys were produced in six male, mongrel dogs by relocating the left kidney of each dog into its neck. Approximately 10 cm of the left jugular vein and left common carotid artery were isolated in the neck of each dog. During the 2.5-hour ischemic interval utilized in the production of a nonfiltering kidney, the kidney was removed from the flank with approximately 4 cm of renal artery and renal vein remaining intact with the kidney. The carotid artery and jugular vein then were ligated distally and clamped with cardiovascular clamps proximally. The artery and vein were severed near the distal ligation. Anastomoses then were made between the carotid artery and renal artery and the jugular vein and renal vein using 6-0 Mersilene cardiovascular suture (Ethicon). At all times the kidney was kept moist with sterile, isotonic saline. A pouch was formed for the kidney by surgically separating the epidermis from its connective tissue. Following completion of the 2.5-hour ischemic period, the clamps were removed and blood flow returned to the kidney. Any bleeding from the anastomoses was allowed to clot completely before the wound was closed. The kidney was placed in the pouch, and both the neck and flank incisions were closed and the dog allowed to recover.

On day 3, a bilateral adrenalectomy was performed via a midline incision, again utilizing sterile technique. At this time, the intact filtering kidney also was removed. Glucocorticoids were replaced with 50-mg injections of prednisolone sodium succinate (Solu-Delta-Cortef; Upjohn) both following adrenalectomy and prior to anesthetizing the dog for experimentation.

On day 4, experiments were conducted on the denervated, nonfiltering kidney. The neck incision was reopened and the renal artery and vein were isolated. Control blood samples were collected and furosemide (5 mg/kg, iv) then was injected. Arterial and venous samples were collected 5, 10, and 20 minutes after furosemide injection.

EXPERIMENT D: EFFECT OF d- OR d,l-PREPRANOLOL ON FUROSEMIDE-INDUCED RENIN RELEASE IN THE DENERVATED, NONFILTERING KIDNEY

In seven dogs, denervated, nonfiltering kidneys again were produced and prepared for experimentation as described in experiment C. Following the collection of control samples, d- or d,l-prepranolol was injected in a priming dose (1 mg/kg, iv) and maintained with an infusion of 1 mg/kg per hour. Three dogs received the d-isomer of prepranolol and four dogs received the d,l-mixture of prepranolol. Twenty-five minutes again were allowed for equilibration and two blood samples were obtained at 10-minute intervals. Furosemide (5 mg/kg, iv) then was injected and blood samples once more were collected at 10-minute intervals.

ANALYTICAL AND STATISTICAL PROCEDURES

Hematocrit was determined on all arterial blood samples by the micromethod. Arterial and renal venous plasma samples and urine samples were analyzed for inulin concentration by the diphenylamine method described by Walser et al.13 Renin activity of arterial and renal venous plasma was determined by radioimmunoassay for the generated angiotensin I.14

Blood pressure and renal blood flow were determined directly from the oscillograph recordings, and the renal plasma flow was calculated from the renal blood flow and hematocrit. Renal resistance was computed as the quo-
tient of the systemic blood pressure and renal blood flow. Renin release was calculated as the product of the renal plasma flow and the renal venous-arterial renin activity difference and was expressed as nanograms released per minute. All values shown are the averages of the two sets of samples drawn 10 minutes apart during each treatment period.

Results of experiments A, B, and D were analyzed by a two-way analysis of variance, and mean differences were determined by the Student-Neuman-Keuls test. Log plots were utilized to express renin release since significant heterogeneity of variance existed between dogs. The design of experiment C allowed analysis by a paired t-test. The 0.05 level of probability was used as the criterion of significance.

Results

EXPERIMENT A: EFFECT OF d-PROPRANOLOL ON FUROSEMIDE-INDUCED RENIN RELEASE IN THE FILTERING AND NONFILTERING KIDNEY

In these six dogs, treatment with d-propranolol decreased renin release in the filtering kidney from a mean control value of 1,687 ± 259 ng/min to 912 ± 372 ng/min (Fig. 1), whereas renal resistance was increased due to a decrease in renal blood flow (Table 1). Subsequent administration of furosemide increased renin release to 1,845 ± 495 ng/min (Fig. 1) and renal resistance remained unchanged (Table 1).

In the nonfiltering kidney, d-propranolol again decreased renin release from 430 ± 84 ng/min to 118 ± 39 ng/min (Fig. 1) and decreased renal blood flow, increasing renal resistance (Table 1). After d-propranolol treatment, furosemide did not increase renin release (204 ± 64 ng/min) (Fig. 1). Renal resistance remained unchanged (Table 1).

EXPERIMENT B: EFFECT OF LIDOCAINE ON FUROSEMIDE-INDUCED RENIN RELEASE IN THE NONFILTERING KIDNEY

Treatment of the nonfiltering kidney with lidocaine produced a highly variable response in the rates of renin release. However, after lidocaine, furosemide produced no further change in renin release in five of six dogs (Fig. 2). There was no significant change in renal blood flow, blood pressure, or renal resistance following lidocaine or furosemide (Table 1).

EXPERIMENT C: EFFECT OF RENAL DENERVATION ON FUROSEMIDE-INDUCED RENIN RELEASE IN THE NONFILTERING KIDNEY

In six dogs with denervated, nonfiltering kidneys, furosemide increased renin release from control values averaging 117 ± 80 ng/min to 731 ± 273 ng/min (Fig. 3). This rise in renin release was accompanied by a significant increase in renal blood flow and decrease in renal resistance (Table 1).

EXPERIMENT D: EFFECT OF d- OR d,l-PROPRANOLOL ON FUROSEMIDE-INDUCED RENIN RELEASE IN THE DENERVATED, NONFILTERING KIDNEY

In seven animals with denervated, nonfiltering kidneys, the effect of d- or d,l-propranolol was studied on furosemide-induced renin release. In six of seven dogs, d- or d,l-propranolol decreased renin release, whereas in the seventh dog there was very little change in renin release (Fig. 4). Propranolol produced no change in renal blood flow or renal resistance from the control value (Table 1). Furosemide produced either a decrease or no significant change in renin release in all seven animals (Fig. 4) and did not produce a change in renal blood flow or renal resistance (Table 1).

Discussion

The control of renin release involves a vascular mechanism in the afferent glomerular arteriole, a tubular mechanism in the distal nephron and a sympathetic, adrenergic mechanism. To evaluate the role of the macula densa receptor, baroreceptor, and sympathetic nervous system in the control of renin release, each mechanism must be isolated. Blaine and co-workers separated the tubular and vascular sites by the use of the nonfiltering kidney model. In the nonfiltering kidney, sodium delivery to the macula densa is eliminated. Thus, changes in filtered sodium load or transport of sodium (chloride) at the macula densa are effectively excluded as factors in the control of renin release. In the nonfiltering kidney, renin release may
TABLE 1  Effect of Treatment with Propranolol (P), Lidocaine (L), and Furosemide (F) on Renal Hemodynamics in the Filtering Kidney, Nonfiltering Kidney, and Denervated Nonfiltering Kidney

<table>
<thead>
<tr>
<th>Experiment A (n = 6)</th>
<th>Filtering kidney</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BP</td>
<td>C</td>
<td>d-P</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>133 ± 5.8</td>
<td>135 ± 5.4</td>
<td>143 ± 7.1</td>
</tr>
<tr>
<td>RBF</td>
<td>144.6 ± 16.7</td>
<td>120.1 ± 14.2*</td>
<td>123.6 ± 12.7</td>
</tr>
<tr>
<td>RR</td>
<td>1.04 ± 0.17</td>
<td>1.39 ± 0.28*</td>
<td>1.28 ± 0.22</td>
</tr>
</tbody>
</table>

| Nonfiltering kidney |
|---------------------|-----------------|---------|---------|
| BP                  | C               | d-P     | F       |
|                      | 133 ± 5.8       | 135 ± 7.1 | 133 ± 5.8 |
| RBF                 | 101.5 ± 16.2    | 69.2 ± 9.8* | 58.3 ± 7.3 |
| RR                  | 1.76 ± 0.37     | 2.48 ± 0.57* | 3.11 ± 0.78 |

| Nonfiltering kidney |
|---------------------|-----------------|---------|---------|
| BP                  | C               | L       | F       |
|                      | 126 ± 6.3       | 122 ± 6.5 | 119 ± 5.7 |
| RBF                 | 93.9 ± 16.4     | 94.0 ± 21.2 | 82.8 ± 18.8 |
| RR                  | 1.80 ± 0.41     | 2.38 ± 0.88 | 2.77 ± 1.13 |

| Denervated nonfiltering kidney |
|-------------------------------|---------|---------|
| C                             |         | F       |
| BP                            | 124 ± 5.5 | 122 ± 6.7 |
| RBF                           | 134.0 ± 17.5 | 153.6 ± 21.4* |
| RR                            | 1.11 ± 0.19 | 0.95 ± 0.16* |

| Denervated nonfiltering kidney |
|-------------------------------|---------|---------|
| C                             |         | F       |
| BP                            |         |         |
| RBF                           | 95 ± 4.0 | 87 ± 7.9 | 96 ± 6.6 |
| RR                            | 52.4 ± 6.0 | 50.9 ± 6.3 | 58.2 ± 8.9 |

Values are expressed as mean ± SE.
C = control; BP = blood pressure; RBF = renal blood flow; RR = renal resistance expressed as peripheral resistance units.
* P < 0.05.

still be altered by the baroreceptor mechanism or the sympathetic nervous system acting directly on the juxtaglomerular cells or modulating the baroreceptor.

The beta adrenergic antagonist d,l-propranolol decreases renin release.3,16 d,l-Propranolol has both beta adrenergic antagonistic properties that are specific to the l-isomer and membrane-stabilizing properties similar to local anesthetics present in both the d- and l-isomer.17 The effect of the racemic mixture on renin release has been attributed to the l-isomer.18,19 The d-isomer did not reduce the basal rate of renin release in one study16 and failed to block renin release occurring in response to a variety of adrenergic stimuli both in vitro and in vivo.16,18,25 Furosemide stimulates renin release at both the vascular and macula densa receptors.26,27 The increase in renin release and accompanying vasodilation produced by furosemide in the nonfiltering kidney was blocked by d,l-propranolol.9 The present experiments document the previous suggestion4 that this blockade resulted from the membrane stabilizing properties of the d-isomer. d,l-Propranolol significantly decreased renin release (Fig. 1) and increased renal resistance (Table 1) in both the filtering and nonfiltering kidney. Renin release was significantly increased by the diuretic in the filtering kidney following baroreceptor blockade by d-propranolol. Since renal resistance was unchanged, this increase in renin release can be attributed to the effect of the diuretic on the functional macula densa mechanism. In the nonfiltering kidney, the effect of furosemide on renin release was blocked following d-propranolol. Similarly, after d-propranolol, furosemide did not change renal resistance (Table 1).

In order to confirm that the membrane-stabilizing properties of d-propranolol can inhibit the furosemide-induced increase in renin release, the local anesthetic lidocaine was infused into the renal artery. The hemodynamic changes following lidocaine were qualitatively similar to those following treatment with d-propranolol. However, the lidocaine-induced increase in renal resistance was not statistically significant due to an increase in variability (Table 1).
similarly, alterations in renin release after lidocaine treatment were highly variable (Fig. 2). These results may suggest differences in the membrane-stabilizing properties of lidocaine and d-propranolol or differences in the binding properties of these drugs to tissue and plasma proteins. Nevertheless, after lidocaine as after d-propranolol treatment, the effect of furosemide on renal hemodynamics (Table 1) and renin release (Fig. 2) was blocked. These data support the previous evidence that the membrane-stabilizing properties of d-propranolol may have the same effect as the racemic mixture on renal hemodynamics and renin release. In addition, to ensure that the d-propranolol was not contaminated with the l-isomer, the specific rotation was measured. The specific rotation of d-propranolol was +25.3°, whereas the specific rotation of the racemic mixture was 0.0°. The specific rotation of d-propranolol has been previously reported as +22.2°. These data indicate that the membrane-stabilizing properties of d-propranolol provide effective blockade of the baroreceptor, resulting in a decrease in renin release. Thus the ability of d,l-propranolol to decrease renin release may be partially due to the membrane-stabilizing activity of the d-isomer.

Several investigators have demonstrated that the sympathetic nervous system may stimulate renin release by a direct action on the JGA. Naughton et al. reported that renal denervation abolished the renin-releasing response of furosemide. It was suggested that neurogenic tone provided a threshold for the direct action of furosemide on the vascular side of the JGA. If this suggestion is correct, furosemide would have no renal hemodynamic effect in the denervated nonfiltering kidney. In this study, adrenalectomy was also performed to eliminate the
primary endogenous source of the catecholamines. Denervation of the nonfiltering kidney was verified by fluorometric determination of norepinephrine levels in three intact kidneys and three transplanted kidneys. Norepinephrine concentration in the intact kidney ranged from 0.04 to 0.25 μg/g of tissue. No norepinephrine was detectable in the denervated nonfiltering kidneys.

Treatment of the denervated nonfiltering kidney with furosemide decreased renal resistance (Table 1) and increased renin release (Fig. 3). These effects were blunted by d- or l-propranolol in six of seven dogs (Table 1; Fig. 4). These data demonstrate that the ability of furosemide to stimulate renin release on the vascular side of the JGA does not require intact sympathetic innervation to the kidneys. Thus, vascular, tubular, and sympathetic adrenergic mechanisms governing renin release are capable of functioning independently.

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