Neural Stimulation of Release of Renin

By Ruben D. Bunag, M.D., Irvine H. Page, M.D., and James W. McCubbin, M.D.

ABSTRACT
Increased vasomotor discharge induced by bleeding caused renal release of renin in anesthetized dogs whether or not there was measurable change in either arterial pressure or total renal blood flow. Release of renin was prevented by ganglion blockade or local anesthesia of the renal nerves. Hemorrhage-induced release of renin occurred more consistently in dogs fed a low-sodium diet than in those fed a standard kennel diet. Stimulation of sympathetic vasomotor discharge by occlusion of the common carotid arteries, while renal perfusion pressure was kept constant, also caused release of renin, as did infusions of norepinephrine, tyramine, or DMPP. Isoproterenol, angiotensin, vasopressin, serotonin, or acetylcholine infused into the renal artery did not cause release of renin. It is concluded that neural stimuli are capable of causing release of renin in the absence of gross change in renal perfusion pressure or flow.

ADDITIONAL KEY WORDS renal denervation hemorrhage renal blood flow dietary sodium sympathetic nervous system carotid occlusion vasomotor discharge anesthetized dogs

It is usually assumed that release of renin during hemorrhage is dependent upon the associated hypotension (1). Since hemorrhage provokes compensatory sympathetic vasomotor discharge, our experiments were designed to investigate whether neurogenic stimulation of the kidney might contribute to release of renin during hemorrhage.

Methods
Adult mongrel dogs of either sex weighing 10 to 25 kg were anesthetized with sodium pentobarbital, 30 mg/kg iv. The left renal vessels were exposed retroperitoneally and a needle inserted into the vein for periodic collection of blood. In most animals, pressures in the brachial and femoral arteries were measured using strain-gauge manometers. Heart rate was recorded with standard ECG leads. To maintain renal perfusion pressure constant during occlusion of both common carotid arteries, umbilical tape was passed around the abdominal aorta above the renal arteries and fashioned into a sliding noose by threading both ends through a narrow glass tube; in these experiments the vagus nerves were cut. Positive-pressure respiration was used and a heating pad kept body temperature constant.

An electromagnetic flowmeter transducer (Carolina Medical Electronics) was placed around the left renal artery in some dogs. The flowmeter was calibrated using whole blood with different hematocrit values; the response was linear over a range of 40 to 400 ml/min. Electrical zero was checked at regular intervals and mechanical zero was obtained at the end of each experiment by occlusion of the renal artery. In the range of 100 to 300 ml/min, the flowmeter could detect a change in flow of 5% with accuracy.

A 20- or 30-ml syringe rinsed with a dilute solution of heparin was used to withdraw blood slowly through a cannula in a femoral vein. After each blood loss (5 ml/kg), 5-ml samples of blood for assay of renin were collected. The total amount of blood lost varied from 15 to 25 ml/kg and it was withdrawn over 15 to 30 min to avoid a decrease in arterial pressure. In experiments in which blood was reinfused, the shed blood to be used was kept in a water bath at 37°C. Blood samples from which plasma was removed for assay were reconstituted with saline and added to the pooled blood to be reinfused.

For assay of renin, 5-ml samples of blood from femoral arteries and renal vein were collected in chilled plastic tubes, mixed with 25 μmole of disodium ethylenediaminetetraacetic acid (EDTA), cooled to 0°C for 2 hours and spun at

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10,000 rpm for 10 min. Plasma supernatants were then divided into three parts, one to serve as the control (unincubated) and the others to be incubated at 37°C for 1 to 4 hours. The renin content of plasma was measured indirectly on pentolinium-treated rats by the amount of angiotensin generated by incubation. Changes in pressor activity equivalent to at least 25% of the assay rat's response to 2 ng of synthetic valylangiotensin II were considered significant. Criteria for establishing that the pressor activity was due to the action of renin on substrate have been described elsewhere (2, 3).

Because dietary sodium has an important influence on the release of renin caused by aortic constriction or infusion of norepinephrine (3), the dogs were divided into two groups to determine if sodium also influenced release of renin in response to hemorrhage. One group of dogs was fed a standard kennel diet (IAMS Dog Food Co., Dayton, Ohio) with a sodium content of 12.5 mEq/100 g for 5 to 14 days before the experiments; the other group was fed a low-sodium diet (Prescription Diet, Hill Packing Co., Topeka, Kansas) with a sodium content of less than 1 mEq/100 g.

**Results**

**Experiments on Dogs Without Restriction of Dietary Sodium**

**Characterization of the Response to Slow Bleeding.** Removal of a total of 15 ml/kg blood caused detectable release of renin in 19 of 28 dogs. In those that released renin, the average arterial pressure was 132 mm Hg before and 133 mm Hg after slow bleeding (Table 1). Arterial pressures remained within 20 mm Hg of the initial levels in all but two. Average heart rate was 149 beats/min before and 200 beats/min after bleeding; it increased in all but 4. Release of renin was detected after removal of 5 ml/kg of blood in 1 dog, 10 ml/kg in 13 and 15 ml/kg in 5. With continued withdrawal of blood beyond the threshold amount there was further increase in release and, with subsequent reinfusion, heart rate and the amount of renin release decreased toward control levels within 30 min. An example is shown in Figure 1.

In the 9 dogs in which release of renin was not detected, average arterial pressure was 137 mm Hg before and 108 mm Hg after removal of 15 ml/kg blood. In 5 of the 9 dogs, the decrease in arterial pressure exceeded 20 mm Hg. Average heart rates were 143 beats/min before and 147 beats/min after bleeding; compensatory cardioacceleration occurred in only 2 of the 9 dogs.

Renal blood flow was recorded in 23 of the 28 dogs. Increased renin release occurred in 14; in 9 of these there was gradual decline in renal blood flow but in the other 5 there was no significant effect. Average renal blood flow before bleeding was 222 ± 35 (sd) ml/min and 183 ± 60 (sd) ml/min after removal of 15 ml/kg blood. In the 9 dogs in which increased renin release was not detected, mean renal blood flow was unchanged in 4 and decreased progressively in 5. Average flows before and after bleeding were 199 ± 48 and 178 ± 52 (sd) ml/min respectively.

To determine the reproducibility of the response to slow bleeding, blood was reinfused into two dogs and, after 30 to 40 min, the same amount of blood was removed a second time. The average arterial pressure was 132 mm Hg before and 127 mm Hg after the first hemorrhage, 138 mm Hg before and 134 mm Hg after the second. Corresponding heart rates were 152 and 183 beats/min after the first hemorrhage, 141 and 172 beats/min after the second. There was no detectable difference in the amounts of renin released by the two hemorrhages.

**Possibility of Splenic Involvement.** To determine if compensatory contraction of the spleen

### Table 1

**Effect of Slow Bleeding (15 ml/kg) on Arterial Pressure, Heart Rate, and Release of Renin in 28 Dogs Fed a Standard Kennel Diet (Figures are Averages and Standard Deviations)**

<table>
<thead>
<tr>
<th>Release of renin</th>
<th>No. of dogs</th>
<th>Arterial pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before bleeding</td>
<td>After bleeding</td>
</tr>
<tr>
<td>Detected</td>
<td>19</td>
<td>132 ± 13</td>
<td>133 ± 20</td>
</tr>
<tr>
<td>Undetected</td>
<td>9</td>
<td>137 ± 10</td>
<td>108 ± 15</td>
</tr>
</tbody>
</table>
in response to hemorrhage might affect the release of renin, two dogs were bled and the blood reinfused; the splenic pedicle was then ligated and 30 min later blood was removed a second time. The average arterial pressure was 126 mm Hg before and 125 mm Hg after the first hemorrhage; corresponding heart rates were 150 and 178 beats/min. Following ligation of the splenic pedicle, the average arterial pressure was 125 mm Hg before and 122 mm Hg after bleeding; corresponding heart rates were 154 and 180 beats/min. There was no detectable difference in the amount of renin released before and after splenic ligation (Fig. 2).

**Neural Involvement in Renin Release.** The relationship between occurrence of renin release and activation of compensatory reflexes, as manifested by tachycardia and steady arterial pressure, suggested that a neural component might be involved in release of renin. This possibility was explored in two ways:

**Effect of ganglion blockade:** Tetraethylammonium chloride (TEAC) produces ganglion blockade with relatively small change in arterial pressure when the total amount is divided into small doses and administered at intervals (4). In 7 dogs that released renin upon removal of 15 ml/kg blood, the blood was reinfused. Intravenous injections of TEAC, 2.5 to 5 mg/kg, were made until a total dose of 25 to 40 mg/kg had been given. Following this, pressor responses to the ganglion-stimulating drug, dimethylphenylpiperazinium (DMPP) (5 to 15 μg/kg), were blocked. The dogs were then bled to the same extent as before treatment with TEAC. Average values for both heart rate and arterial pressure were significantly lower before the second hemorrhage than before the first (Table 2). In 6 of the 7 dogs renin was not released by the second hemorrhage. In the remaining dog, the amount of renin released by the second hemorrhage was approximately half that re-
leased by the first. In all animals, arterial pressures, although initially lower, were maintained at the same level during the second hemorrhage. This suggests the involvement of compensatory mechanisms operating independently of the nervous system, but their nature is not known. In 4 of the 7 dogs, the first hemorrhage was accompanied by compensatory cardioacceleration that did not reappear during the second. Tracings recorded from a dog before, during, and after administration of TEAC, 25 mg/kg, are shown in Figure 3.

**Effect of local anesthesia of the renal nerves:** In 3 dogs that released renin after removal of the usual amount of blood, we infiltrated the renal pedicle with 2 to 4 ml of 2% lidocaine instead of reinfusing the shed blood. After a 15-min wait, amounts of renin in the renal venous blood were reduced to the prebleeding level in all 3 dogs. Average arterial pressure was 137 mm Hg before and 132 mm Hg after lidocaine infiltration. Corresponding average heart rates were 188 and 190 beats/min.

**Experiments on dogs fed a low-sodium diet**

**Release of Renin by Hemorrhage.** Nine dogs were bled (15 ml/kg) in the usual way. Arterial pressure remained steady in all, average values being 132 mm Hg before and 126 mm Hg after bleeding (Table 3). Compensatory tachycardia occurred in 7 of the 9 dogs; average heart rates were 121 beats/min before and 152 beats/min after bleeding. Renin was released in all.

Average renal blood flow was 144 ml/min before and 127 ml/min after bleeding (Table 3). Hemorrhage produced a progressive decrease in renal blood flow in 4 dogs but had no significant effect in the other 5. Since the initial renal blood flow was lower in these animals than in those fed a regular diet, larger random samples of 20 dogs in each group were compared. Dogs fed a regular diet had an average renal blood flow of 246 ± 20.9 (SEM) ml/min compared with 160 ± 6.2 (SEM) ml/min for those fed a low-sodium diet (P < 0.005). Average body weight was 15.4 kg (range 9.7 to 22) for dogs fed the regular diet.

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### Table 2

Effects of Ganglion Blockade with Tetraethylammonium Chloride (TEAC), 25 to 40 mg/kg, on Responses to Hemorrhage (Figures are Averages and Standard Deviations)

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of dogs releasing renin</th>
<th>Arterial pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before bleeding</td>
<td>After bleeding</td>
<td>Before bleeding</td>
</tr>
<tr>
<td>Before TEAC</td>
<td>7</td>
<td>143 ± 12</td>
<td>139 ± 13</td>
</tr>
<tr>
<td>After TEAC</td>
<td>1*</td>
<td>114 ± 8</td>
<td>115 ± 11</td>
</tr>
</tbody>
</table>

*Release reduced by approximately 50%.*

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*Downloaded from http://circres.ahajournals.org/ by guest on November 12, 2017*
and 14.8 kg (range 9.7 to 22) for those fed the low-sodium diet.

Effect of Carotid Occlusion. Increased sympathetic nerve activity was produced in 7 dogs by occluding both common carotid arteries for 20 to 25 min after cutting the vagus-sympathetic-depressor trunks. The abdominal aorta was partially constricted as necessary to maintain renal perfusion pressure constant. Hemodynamic changes (mean and standard deviation) were: heart rate increased from 124 ± 9/min to 160 ± 23; systemic arterial pressure increased from 129 ± 17 mm Hg to 191 ± 28; and, in 5 of the 7 dogs, renal blood flow decreased from 158 ± 43 ml/min to 115 ± 22. Renal blood flow

![Diagram](image URL)

**FIGURE 3**
The effect of ganglion blockade on the renal response to slow bleeding. First panel shows the effect of an initial hemorrhage after which the blood was reinfused. The middle panel was recorded during 5 intravenous injections of tetraethylammonium chloride (TEAC), each 5 mg/kg. The last panel shows the response to slow bleeding 10 min after the last injection of TEAC. Description the same as in Figure 1. Dog, 21.6 kg, male.

**TABLE 3**
Effect of Dietary Sodium on Hemodynamics during Release of Renin by Bleeding (15 ml/kg) (Figures are Averages and Standard Deviations)

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. of dogs</th>
<th>Arterial pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
<th>Renal blood flow* (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before Bleeding</td>
<td>After Bleeding</td>
<td>Before Bleeding</td>
</tr>
<tr>
<td>Regular</td>
<td>19</td>
<td>132 ± 13</td>
<td>133 ± 20</td>
<td>149 ± 16</td>
</tr>
<tr>
<td>Low-sodium</td>
<td>9</td>
<td>132 ± 15</td>
<td>126 ± 21</td>
<td>121 ± 30</td>
</tr>
</tbody>
</table>

*Weight of dogs in the two series was comparable.
†Recorded in 14 dogs.
was unchanged in 1 dog and slightly increased in the other. Renin was released in all animals. Tracings recorded during one of the experiments are shown in Figure 4.

Release of Renin by Adrenergic Drugs. Infusion of norepinephrine causes release of renin (5,6); therefore, experiments were done in 26 dogs to determine if other drugs might also cause its release. Table 4 lists the various drugs tested, the infusion rates used, average renal blood flow before and during infusion, and whether release of renin occurred. All infusions were directly into a renal artery and were given in normal saline solution at a rate of 0.36 ml/min. In addition to norepinephrine, only two other agents, tyramine and DMPP, caused release of renin. These drugs were also effective when given by intravenous infusion while renal perfusion pressure was kept relatively constant by aortic constriction. The second panel of Figure 4 shows that renin was released by intravenous infusion of DMPP.

Discussion

Huidobro and Braun-Menendez (1) showed in 1942 that extensive hemorrhage results in release of renin into the circulation, and they attributed the release to the accompanying hypotension. Our results indicate that at least one additional mechanism must be involved, because in some experiments renin was still released when there was no decrease in either renal arterial blood pressure or renal blood flow following hemorrhage. It appears that the mechanism is neural in nature since it was made inoperative by ganglion blockade or by infiltration of a local anesthetic into the region of the renal hilus. The release is probably mediated by the sympathetic portion of the nervous system since there was a rough correlation between occurrence of compensatory tachycardia and release of renin. Supporting this suggestion is the finding that sympathetic discharge provoked by carotid occlusion also caused release of renin. The mild nature of the stimulus elicited by small, nonhypotensive hemorrhages makes plausible the suggestion made long ago by Braun-Menendez et al. (7) that “... renin is one of the many physiologic substances which the body uses to maintain its homeostasis.” Brown et al. (8) have recently confirmed that large hemorrhages result in release of renin but they were unable to detect its release after small ones. It is unlikely that neural transmission to the kidney maintains essential hypertension or hypertension due to nephritis since Page and Heuer (9,10) found renal denervation to be ineffective in lowering pressure. There remains the possibility that a mechanism of this type might play a part in initiating the hypertension.

The ultimate nature of the stimulus, or stimuli, that causes release of renin remains unknown. In these experiments involving neural stimulation, it appears not to depend upon gross change in renal perfusion pressure or flow, although there may well be regional changes in flow and pressure, especially since Carriere et al. (11) have reported that hemorrhage produces a pattern of renal cortical flow similar to that produced by electrical stimulation of the renal nerves, and Vander (5) has shown that this type of stimulation causes release of renin. As another alternative, since the juxtaglomerular cells are richly supplied with nonmyelinated nerves (12,13), the neural mechanism might involve a direct effect upon these cells.

Vander (5) and Wathen et al. (6) have shown that infusion of norepinephrine causes release of renin. We have confirmed this finding and found that tyramine and DMPP are also effective in causing release. A number of other vasoactive drugs were ineffective. Tyramine acts in part by releasing norepinephrine and DMPP by sympathetic ganglion stimulation; this suggests a common mechanism based on their sympathomimetic properties. Whether release of renin stimulated by endogenous catecholamines of adrenomedullary origin participates in the response to hemorrhage is not known.

It has now become clear that many factors are involved in release of renin. Decrease in renal perfusion pressure, change in glomera-
Release of renin caused by carotid occlusion and infusion of DMPP. Numbers on top indicate the clock time. To maintain renal perfusion pressure (femoral arterial blood pressure) at approximately pre-occlusion level, the abdominal aorta was constricted as needed. Dog, 16.5 kg, male. Fed low-sodium diet for 8 days prior to experiment; urinary sodium excretion 3.8 mEq/24 hours.

**TABLE 4**

Effects of Drugs Infused into a Renal Artery on Renal Blood Flow and Release of Renin

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of infusions</th>
<th>Infusion rate (µg/kg/min)</th>
<th>Average renal blood flow (ml/min)</th>
<th>Release of renin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>9</td>
<td>0.05-0.1</td>
<td>136</td>
<td>Detected</td>
</tr>
<tr>
<td>Tyramine</td>
<td>6</td>
<td>10-20</td>
<td>159</td>
<td>Detected</td>
</tr>
<tr>
<td>DMPP</td>
<td>4</td>
<td>5-10</td>
<td>138</td>
<td>Detected</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>4</td>
<td>0.1-0.2</td>
<td>149</td>
<td>Undetected</td>
</tr>
<tr>
<td>Angiotensin</td>
<td>4</td>
<td>0.02-0.05</td>
<td>176</td>
<td>Undetected</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>3</td>
<td>1-5</td>
<td>127</td>
<td>Undetected</td>
</tr>
<tr>
<td>Serotonin</td>
<td>2</td>
<td>20-40</td>
<td>180</td>
<td>Undetected</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>5</td>
<td>1</td>
<td>112</td>
<td>Undetected</td>
</tr>
</tbody>
</table>

*Doses for norepinephrine and isoproterenol are expressed in terms of the base; all others are in terms of the commercially available salts.*

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lar filtration, decrease in blood volume, and change in sodium balance are among those that may result in change in renal intramural tension, neural impulses, or electrolyte environment. When dogs had been on a low-sodium diet, release of renin in response to either hemorrhage or infusion of norepinephrine was much more consistent. Since total renal blood flow was initially lower in these dogs than in those fed a standard kennel diet, this may have been a factor related to the ease with which release of renin could be stimulated. Vander and Miller (14) have presented a strong argument for change in tubular transport of sodium as the final stimulus for release of renin; we have suggested (3) that the state of sodium balance may instead condition the sensitivity of response of the renin-releasing mechanism. No answer is as yet available and the results of the present experiments, like nearly all previously published ones, are consistent with either explanation.

References
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