Effect of Hemorrhage on Arterial Plasma Renin Activity in the Rabbit


ABSTRACT

Arterial plasma renin activity in the rabbit was measured at the beginning and end of a brisk hemorrhage and 40 min later. Activity rose to a mean of two and one-half times the control value during the hemorrhage, and was five times greater than the control 40 min later. The rise in plasma renin activity was not due to anesthesia, as light pentobarbitone produced no change. Conscious rabbits, severely bled from a carotid cannula, 2 to 5 hr after an ether anesthetic, also showed an increase in plasma renin activity at the end of hemorrhage. Forty-eight hours after both kidneys had been removed, renin activity was virtually absent and did not rise after hemorrhage.

ADDITIONAL KEY WORDS kidney enzyme blood pressure anesthesia removal of both kidneys vasoconstrictor activity

Huidobro and Braun-Menéndez showed in 1942 that blood loss seemed to raise plasma renin level in the dog. Hamilton and Collins and Sapirstein et al. produced evidence for a similar finding. Ziegler and Gross found that the rise of arterial pressure produced in rats 24 hr after nephrectomy by cross circulation was greater if the donor rat had been bled. Increased angiotensin levels have been found in dog plasma after severe hemorrhage.

Much evidence now accumulated implicates the renin-angiotensin system in stimulating aldosterone production. Laragh et al. and Genest et al. have shown that infusions of angiotensin, at rates insufficient to raise systemic blood pressure, can increase aldosterone output, especially in sodium-depleted subjects. Thus, the increase in aldosterone secretion that occurs after hemorrhage and is prevented by nephrectomy, also suggests that renin output may be increased after hemorrhage.

Lever and Robertson could find no more than a 50% rise in plasma renin-like activity between the first and last of four 20-ml blood samples removed in 2 to 5 min, from the carotid artery of the conscious rabbit, after recovery from ether anesthesia. The early experiments made use of methods of assay which were unsatisfactory or possibly nonspecific. Those of Lever and Robertson required large volumes of blood such as to increase the plasma renin activity over the course of the initial control sample. Using a new, more sensitive method for measuring plasma renin-like activity, we have studied the effect of hemorrhage in rabbits.

Methods

1. Eleven adult rabbits weighing 2.5 to 4.0 kg were lightly anesthetized with 25 to 35 mg/kg of pentobarbitone sodium. After infiltrating the neck with lignocaine and epinephrine, both carotid arteries were cannulated and the animal was given 5,000 units of heparin. From one cannula the blood pressure, close to mean blood pressure, was recorded using a mercury manometer with a direct-writing float (Condon type*). The other cannula was used to bleed the animal rapidly; 12 to 16 ml/kg was removed in 2 to 5 min. The initial 20-ml sample was used for the first estimations and the final 12 to 20 ml, used for the second estimations, was taken when blood pressure had returned to normal. After hemorrhage the animal was allowed to recover for 40 min. From one cannula 20-ml samples were taken and used for the second estimations. From the other cannula the rabbit was allowed to recover for 40 min. From one cannula 20-ml samples were taken and used for the second estimations.

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was lowest. After a further 40 min, during which the blood pressure was allowed to rise, further samples were taken and the animal was killed (fig. 1).

2. Both kidneys were removed from four rabbits under thiopentone and lignocaine anesthesia. Either 24 or 48 hr later, samples were collected as in (1), at the beginning and end of hemorrhage of 15 ml/kg during 5 min.

3. Central ear arteries of five adult rabbits were cannulated with polyethylene tubing using lignocaine as a local anesthetic; the tubes were kept patent by filling with heparin. Twenty-four hours later two 6-ml samples were taken from each conscious rabbit at half-hour intervals. Each sample took about 4 min to collect and was simultaneously replaced by a similar volume of 0.9% sodium chloride injected into the marginal ear vein. In some rabbits systolic blood pressure readings were taken from the opposite central ear artery using a Grant-Rothschild capsule. The animals were next lightly anesthetized with 25 to 30 mg/kg of pentobarbitone sodium which was maintained at a depth barely sufficient to prevent responses to painful stimuli. Two further ear artery blood samples were then taken, 30 min and 1 hr after inducing the anesthestia.

4. Polyethylene cannulae were inserted into one carotid artery in 11 adult rabbits under ether anesthesia and the tubes were brought out subcutaneously at the back of the neck. After allowing 2 to 5 hr for blood pressure to rise, the animals were bled 20 to 30 ml/kg (60 to 112 ml). Samples were taken at the beginning and end of hemorrhage. Blood pressure was measured with the Condon manometer before bleeding commenced.

The assay of plasma renin-like activity is fully described elsewhere in this issue. In this laboratory renin measurements have always been expressed in terms of the activity of a standard ethanol-dried rabbit-kidney powder. As most enzymes are measured in terms of the rate of the reaction they catalyze, and as standard angiotensin preparations are available, we have standardized the old unit in terms of the rate of angiotensin production. The renin unit is that amount which, under conditions of substrate excess, will produce the pressor equivalent of 1.8 μg of angiotensin II-β-amide per hr at pH 6.0 in 0.1 M phosphate buffer using rabbit renin substrate.

Results

1. HEMORRHAGE IN THE ANESTHETIZED RABBIT

Table 1 shows the results of 11 experiments. Plasma renin-like activity rises from a mean of 2.8 units/L (±0.79 se) to 6.9 units/L (±1.15 se) at the end of hemorrhage and rises further to a mean of 12.3 units/L (±2.35 se) 40 min later. The increments of renin values at the end of hemorrhage compared with the control values were significant ($P < .001, n = 9, \text{Students} \text{"}t\text{"} \text{test}$) as were the increments of values after 40 min compared with control ($P < .001, n = 11$). Typical rates of angiotensin formation for a single experiment are shown in figure 2.

Thus, the arterial plasma renin activity rose rapidly to between two and three times the control value by the end of a severe 2 to 5 min hemorrhage, and were between four and five times control values 40 min later. A typical mean blood pressure recording is shown in figure 1. The lowest pressure varied from 25 to 40 mm Hg. The rise in blood pressure varied; it did not rise above 20 mm Hg in one animal and in two others rose over 80 mm Hg at 40 min. The final renin values tended to show an inverse correlation with the height of the final mean blood pressure. ($r = .60, n = 10, .05 < P < .1, \text{experiment 4 omitted as unpublished results suggest defective renin release at this blood pressure.}$)

It seems possible that when the blood pressure after hemorrhage rose toward normal, the stimulus to renin output was 'turned off.' In experiments 6, 7, 10 and 11 in table 1, the final renin value was close to the value found at lowest blood pressure. In these experiments the mean blood pressure was 80 mm Hg or above at the time of the final sample in all except experiment 6. At the same time, the hematocrit fell from an average of 34 to 29.5

![Blood pressure record in a typical hemorrhage experiment in the anesthetized rabbit with times of sampling indicated.](http://circres.ahajournals.org/DownloadedFrom/31x55to565x787)
between the first and last samples. This dilution might mean that for any given renin output, the final samples would have a lower concentration of renin activity than the samples at lowest blood pressure.

For these reasons, perhaps, there was more variation in the final samples than in the samples at the time of lowest blood pressure. Samples at the time of lowest blood pressure were therefore used in other experiments as being more reliable.

2. HEMORRHAGE IN RABBITS FOLLOWING THE REMOVAL OF BOTH KIDNEYS

In 11 of 12 rabbits without kidneys for 48 hr, no renin-like activity was found in the plasma; in the other, 1 unit/L was present. When four of these nephrectomized rabbits were bled there was no rise in plasma renin-like activity.

When incubated with angiotensin for 72 hr there was no evidence of angiotensinase in the plasma extracts from nephrectomized animals. When incubated alone for the same length of time, no pressor or depressor activity appeared in these extracts. Thus the failure to find a rise in renin-like activity was not due to altered angiotensinase or other factors appearing in the nephrectomized rabbit plasma. The results are shown in table 2.

3. EFFECT OF PENTOBARBITONE ANESTHESIA ON PLASMA RENIN ACTIVITY

Table 3 shows the results of five experiments. There was no significant difference (.3 < P < .4) in plasma renin-like activity.

![Graph](https://example.com/graph.png)

**FIGURE 2**

Rate of angiotensin formation in a typical hemorrhage experiment, showing control, "trough" (or lowest blood pressure) and 40 min values. Forty minute estimations are in duplicate.

**TABLE 1**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control</th>
<th>At lowest B.P. (2 to 5 min)</th>
<th>40 min</th>
<th>Before</th>
<th>40 min</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>unit/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.4</td>
<td>10.0</td>
<td>80</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.1</td>
<td>15.2</td>
<td>100</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>13.5</td>
<td>85</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.4</td>
<td>10.0</td>
<td>90</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.7</td>
<td>13.3</td>
<td>90</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.0</td>
<td>8.7</td>
<td>100</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3.6</td>
<td>6.7</td>
<td>80</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>9.3</td>
<td>34.2</td>
<td>70</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.4</td>
<td>11.2</td>
<td>105</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5.6</td>
<td>6.7</td>
<td>80</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1.8</td>
<td>6.0</td>
<td>105</td>
<td>85</td>
<td></td>
</tr>
</tbody>
</table>

Mean 2.8 ±0.79 se 6.9 ±1.15 se 12.3 ±2.35 se

Rise 4.1 vs. control 9.6 vs. control

Significance t 7.07 <.001

Significance P <.001

**TABLE 2**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>N 1</th>
<th>N 2</th>
<th>N 3</th>
<th>N 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renin Activity (Unit/L)</td>
<td>&lt;0.5</td>
<td>1.2</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Lowest Blood Pressure</td>
<td>&lt;0.5</td>
<td>1.2</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>
TABLE 3

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Conscious</th>
<th>Anesthesia</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.0, —</td>
<td>2.8, 2.9</td>
<td>—5</td>
</tr>
<tr>
<td>2</td>
<td>5.2, 7.3</td>
<td>10.7, 5.0</td>
<td>+23</td>
</tr>
<tr>
<td>3</td>
<td>2.6, 2.4</td>
<td>2.6, —</td>
<td>+4</td>
</tr>
<tr>
<td>4</td>
<td>1.5, 1.4</td>
<td>1.8, 1.6</td>
<td>+18</td>
</tr>
<tr>
<td>5</td>
<td>1.1, 1.3</td>
<td>1.0, 1.2</td>
<td>—9</td>
</tr>
<tr>
<td>Mean</td>
<td>2.9</td>
<td>3.3</td>
<td>+6.2</td>
</tr>
</tbody>
</table>

*Four single samples at half hour intervals in each experiment; anesthesia induced immediately after the second sample.

between the conscious and anesthetized state in the same rabbits. It can also be seen that the mean value of 2.9 units/L is little different from the mean of 2.8 units/L for the 11 anesthetized rabbits in the hemorrhage experiments (table 1). When satisfactory readings were obtained with the ear capsule, systolic blood pressure did not appear to be altered by the anesthetic.

4. HEMORRHAGE IN THE CONSCIOUS RABBIT

In the conscious rabbit (2 to 5 hr after the preparatory operation under ether), plasma renin-like activity at the end of a large hemorrhage (20 to 30 ml/kg) was always greater than at the beginning of the hemorrhage (table 4). There was a mean rise of 2.9 units/L compared with 4.1 units/L in anesthetized rabbits. However, because of the much greater variation, these results are significant only at the 1% level (n = 11, "t" = 2.7, P = 0.01). Mean control value was higher (3.8 units/L) than in the anesthetized rabbits (2.7 units/L) or the conscious rabbits with ear artery cannulae (2.9 units/L). Mean blood pressure was measured more than once on several rabbits after the ether anesthetic, and in all rabbits immediately before hemorrhage. It was never below 75 mm Hg and varied between this and 120 mm Hg.

**Discussion**

These results show not only a large but a rapid increase in plasma renin-like activity as a result of hemorrhage in anesthetized rabbits. This rise is too great to be accounted for by changes in blood volume. Our results differed from those of Lever and Robertson and several possibilities might account for this. The possibility that renin levels are altered by anesthesia has been explored, but light pentobarbitone anesthesia produced no detectable change. However, it remained possible that conscious rabbits react in a different way to blood loss than anesthetized rabbits. For this reason, the experiments of Lever and Robertson were repeated as exactly as possible.

There was a significant rise once again in renin-like activity in the conscious rabbit after bleeding. However, the level of significance was much less than with anesthetized animals. The post-operative state of the rabbit, and possibly the ether anesthetic might produce a variation in renin activity, thus obscuring the rise caused by the test hemorrhage.

It could be argued that hemorrhage causes release of a substance with renin-like activity from sites other than the kidney or activates some inactive precursor in the plasma. Our results in nephrectomized rabbits, like those of others, exclude an extrarenal origin of renin and show that the precursor of renin must also come from, or be dependent on, the kidney.

Hemorrhage initiates many mechanisms for the maintenance of effective blood flow to the brain and heart. It has been shown that blood levels of catecholamines increase within 5 to
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10 min after a severe hemorrhage,15 that aldosterone output increases within 15 min,16 and that plasma vasopressin concentration also increases after hemorrhage.17 Plasma levels of ACTH and glucocorticoids also increase after hemorrhage, and all these substances may alter renal function and possibly the output of renin. Scornik and Paladini6 have shown that norepinephrine infusion raises angiotensin blood levels in the dog.

Besides these indirect effects, the direct lowering of renal arterial perfusion pressure has been shown to have an effect on output of renin-like material.18 It is thus not surprising that hemorrhage should produce a large increase in plasma renin-like activity. The rapidity of the response suggests that renin output may be stimulated by a direct intrarenal mechanism or by the action of the sympathetic nervous system.

References

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JOHN K. MCKENZIE, MICHAEL R. LEE and WILLIAM F. COOK

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