Changes in Hypertensinase and Hypertensinogen Following Nephrectomy

By DEAN A. COLLINS, PH.D., M.D., AND CONCETTA D. HARAKAL, M.S.

The hypertensinase content of dog's plasma is decreased 48 hours after nephrectomy. This decrease may contribute to the increased blood pressure response to renin shown by nephrectomized animals. When plasma is incubated with a relatively small concentration of renin, the yield of hypertensin increases after nephrectomy. This finding correlates with decreased hypertensinase and the increased pressor response to renin characteristic of the postnephrectomy state. Mean plasma hypertensinogen shows about a threefold increase, and its significance is discussed.

WHILE most investigators have found that nephrectomized animals give increased pressor responses to renin, a few workers have disagreed. In order to eliminate possible variables in the intact animal we have performed in vitro experiments on plasma taken from dogs before and 48 hours after nephrectomy. The plasma was incubated with renin under standard conditions and the amounts of hypertensin (angiotensin) resulting determined. The smallest concentration of renin compatible with reliable determinations was used in order to approximate as closely as possible the renin concentrations produced in the plasma of animals when they are injected with the usual test doses of this enzyme.

A greater concentration of hypertensin resulted from incubation of renin with plasma from nephrectomized dogs than with that from normal dogs. The increase in renin-substrate (hypertensinogen) in plasma after nephrectomy is a possible factor contributing to the greater yields of hypertensin. This factor cannot be eliminated. However, a decrease in hypertensinase (angiotonase) in plasma, by diminishing the rate of hypertensin destruction during the incubation, is also capable of increasing the yield of hypertensin. Munoz and colleagues reported that hypertensinase is diminished after nephrectomy but later stated that their results were invalidated by hemolysis. Dexter concluded that the concentration of hypertensinase in plasma is normal 48 hours after nephrectomy. Since his study involved only 4 nephrectomized dogs compared with different normal dogs, we have determined hypertensinase in the plasma of 10 dogs and have repeated the assays 48 hours after nephrectomy. All results have been expressed in the units defined by Braun-Menendez and colleagues.

PROCEDURE

Plasma was obtained from the arterial blood of unanesthetized dogs. In the determination of hypertensinase, citrate was used as an anticoagulant; in all other procedures heparin, 0.01 mg. (1 unit) per milliliter of blood, was employed. Bilateral nephrectomy was performed on the dogs under pentobarbital anesthesia using aseptic operative technique.

Assay of Hypertensin. Hypertensin was measured and calculated by a modification of the method described by Braun-Menendez and associates. Cats were anesthetized with Dial and urethane (Ciba), 0.88 ml. per kilogram intraperitoneally. Heparin or Paritol A was used as an anticoagulant in the manometer system. Samples at about pH 7.4 were injected into the exposed femoral vein in doses of 2 ml. or less to minimize effects on blood volume. Responses from the unknown and the hypertensin reference standard† were

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made nearly equal and kept between 20 to 35 mm. Hg. Several comparisons were made, often six or more. The largest number of comparisons was possible in the hypertensinase assays where ample samples were possible.

Incubation of Plasma with Renin. The amount of hypertensin present after the incubation of plasma with renin was determined. The plasma was adjusted to pH 7.4 (glass electrode) and 0.5 M phosphate buffer at pH 7.4 was added in a concentration of 1:20. A sufficient number of stoppered tubes, each containing 9.6 ml. buffered plasma and 2.8 units hypertensinase-free renin in a volume of 0.4 ml., were incubated at 37 C. for 8, 11 or 15 minutes. The pH variation was within 0.05. The reaction was stopped by acidifying to about pH 5.2 and heating in boiling water for six minutes. After centrifugation the supernatant fluid was evaporated to one-half or one-fourth of its original volume and assayed for its hypertensin content. Controls were made by substituting Ringer's solution or heat-inactivated renin for the renin solution. A few experiments were discarded because the control samples had significant effects on blood pressure. Recovery experiments demonstrated that acidification and heating resulted in no loss of hypertensin.

Dog renin was used and consisted of fraction B of the method of Schales. The hypertensinase in this fraction was destroyed by incubation at pH 3.9 for 20 minutes at 37 C. The material at pH 7 was lyophilized and stored in a vacuum desiccator. This renin had a potency of 14 units per milligram.

The above procedure was performed on the plasma of normal dogs about two weeks before nephrectomy. In order to obtain sufficiently large samples for bio-assay, blood equal to about 10 ml. per kilogram of body weight was taken by arterial puncture. The procedure was repeated on the same dogs 48 hours after nephrectomy. Larger samples were possible for these determinations since the animals were sacrificed.

Assay of Renin. Renin preparations were assayed by a method based on that described by Braun-Menendez and co-workers. Substrate (hypertensinogen) was furnished by heparinized dog plasma. Its hypertensinase was destroyed by the method of Dexter, Haynes and Bridges. After the dialysis involved in this procedure the plasma was restored to its original volume by evaporation in cellophane tubes in front of a fan, and the pH was adjusted to 7.6 (phenol red). Absence of hypertensinase was demonstrated by incubation of the plasma with hypertensin, and an adequate content of substrate was assured by determination of hypertensinogen. A number of tubes were prepared, each containing the following mixture: 8.0 ml. substrate, 0.15 ml. 1:1000 Merthiolate (Lilly), 0.5 ml. 0.5 M phosphate buffer (pH 7.6), varying amounts of renin solution, water ad 10 ml. Control mixtures containing heat inactivated renin were included. The tubes were incubated for two hours at 37 C., and the reaction was stopped by acidification and heating (see above). After centrifugation the supernatant fluid was reduced to one-fourth of its original volume by evaporation and the material assayed for hypertensin. One-half unit of hypertensin was considered to be produced by one unit of renin. The amount of renin added per tube was less than one unit, making hypertensin production less than 0.5 unit (0.05 unit per milliliter). Under the above conditions the relation between renin concentration and hypertensin formation is said to be linear for values between 0.005 to 0.05 unit of hypertensin per milliliter.

Assay of Hypertensinogen. Hypertensinogen was measured by the procedure described by Braun-Menendez and associates except that the mixtures were not diluted after incubation and the reaction was terminated by acidification and heating. The supernatant fluid was reduced to half its original volume and assayed for hypertensin. The renin used in this determination was fraction C of Schales's method and was made from desiccated and defatted hog kidney cortex (at 37 C.) obtained from the Viobin Corporation. The preparation was lyophilized after its hypertensinase had been destroyed by exposure to pH 3.9 for 20 minutes at 37 C.

Assay of Hypertensinase. Hypertensinase was determined and calculated by the method described by Dexter with only minor alterations. The precautions recommended for sterility were followed. The degree of hemolysis
Table 1.—Yield of Hypertensin from Incubation of Plasma Mixture Containing 0.28 Unit per Milliliter of Renin

<table>
<thead>
<tr>
<th>Dog</th>
<th>Before Nephrectomy</th>
<th>48 Hours After Nephrectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>units/ml</td>
<td>units/ml</td>
</tr>
<tr>
<td></td>
<td>8 min. 11 min. 15 min.</td>
<td>8 min. 11 min. 15 min.</td>
</tr>
<tr>
<td>1</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>2</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>3</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
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<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>5</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>6</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>7</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>8</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>9</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>10</td>
<td>0.04</td>
<td>0.07</td>
</tr>
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</table>

Table 2.—Plasma Hypertensinogen before and 48 Hours after Nephrectomy

<table>
<thead>
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<th>Before</th>
<th>After</th>
</tr>
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<tr>
<td></td>
<td>units/ml</td>
<td>units/ml</td>
</tr>
<tr>
<td>5</td>
<td>0.4</td>
<td>2.2</td>
</tr>
<tr>
<td>6</td>
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<td>1.4</td>
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<tr>
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<td>1.5</td>
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<td>1.8</td>
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<tr>
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<td>0.7</td>
<td>2.2</td>
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<tr>
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<td>0.5</td>
<td>2.7</td>
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<td>11</td>
<td>0.2</td>
<td>1.3</td>
</tr>
<tr>
<td>12</td>
<td>0.4</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Table 3.—Plasma Hypertensinase before and 48 Hours after Nephrectomy

<table>
<thead>
<tr>
<th>Dog</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>units/ml</td>
<td>units/ml</td>
</tr>
<tr>
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<td>2.4</td>
<td>1.8</td>
</tr>
<tr>
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<td>2.1</td>
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<td>18</td>
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<tr>
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<tr>
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<td>1.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

was evaluated by determination of plasma hemoglobin by the method of Chornock and Karr rather than by the method used by Dexter. Plasma with more hemoglobin than 7 mg. per 100 cc. was discarded. Before assay for hypertensin, samples were evaporated to one-fourth of their original volume. The amounts of plasma added to the mixtures were such that 30 to 60 per cent of the hypertensin was destroyed during incubation.

Preparation of Hypertensin.* Most of the hypertensin used in this study was prepared by the method described by Braun-Menendez and colleagues.1 A more potent preparation made by a method devised in this laboratory (unpublished) was used in a few experiments. After lyophilization the preparations were compared with a reference standard.

RESULTS

Incubation of Plasma with Renin. When normal plasma was incubated with 0.28 unit of renin per milliliter, the average amounts of hypertensin found per milliliter were 0.03 unit after 8 minutes and 0.05 unit after 15 minutes of incubation (table 1, dogs 1 to 5). These amounts were small compared with the maximum possible yield, calculated as 0.48 unit per milliliter. Forty-eight hours after nephrectomy the average yields in the same animals were increased to about 0.07 unit per milliliter at 8 minutes and 0.1 unit per milliliter at 15 minutes. Somewhat smaller increases occurred in experiments in which the incubation time was 11 minutes (table 1, dogs 6 to 10). This phenomenon may be analogous to the greater responses in blood pressure to renin seen in the intact animal after nephrectomy.

Hypertensinogen. Hypertensinogen was determined in dogs 5 to 10 listed in table 1 and in two additional animals. Data were corrected for dilution by the anticoagulant. The average concentration rose from about 0.07 unit per milliliter to 1.8 units per milliliter as a result of nephrectomy (table 2).

Hypertensinase. A decrease in hypertensinase following nephrectomy, by diminishing the amount of hypertensin destroyed during the incubation, could produce increases in the yields of hypertensin such as those observed in the previous experiments. Consequently hypertensinase was determined in the plasma of 10 dogs before and 48 hours after nephrectomy. In every animal there was a fall, varying from about 16 to 60 per cent (table 3). The average

* We are greatly indebted to Dr. O. M. Helmer of Eli Lilly and Company for a generous sample of angiotensin.
concentration for the normal state was 2.1 units per milliliter and that after nephrectomy 1.4 units per milliliter. Two dogs were subjected to the surgical procedures involved in nephrectomy without actual removal of the kidneys; plasma hypertensinase did not decrease.

Owing to the short period of incubation of plasma with renin, it was questionable whether the action of hypertensinase was great enough to influence hypertensin concentration. The problem was studied by incubating hypertensin (0.1 unit per milliliter) in a mixture of plasma identical with that previously used except that no renin was included. The reaction was stopped after 11 minutes by acidification and heating. Destruction of hypertensin was determined by comparison of the samples with identical, unincubated mixtures. This experiment was done on the plasmas of seven normal dogs and of six different nephrectomized animals. While it was difficult to detect any destruction of hypertensin by the plasma of nephrectomized dogs, an average of about half of the hypertensin was destroyed by normal plasma. This result showed that the action of hypertensinase was significant and that the fall in hypertensinase was at least partly responsible for the increased amounts of hypertensin obtained in the incubation of plasma with renin after nephrectomy.

**DISCUSSION**

Our data show an average fall of 34 per cent in plasma hypertensinase after nephrectomy. While this fall is a significant factor in our in vitro experiments, its importance in the intact animal cannot be accurately assessed. While the effect of a moderate dose of renin may persist as long as 30 minutes, the maximum elevation in blood pressure is reached in about two minutes. Our incubations of hypertensin with plasma involve periods of 11 minutes. However the authors believe that there is a strong possibility that the fall in hypertensinase is significant in the greater and more prolonged response to renin seen in the nephrectomized animal. Gollan, Richardson and Goldblatt have shown that a twofold increase in plasma hypertensinase markedly reduces or abolishes the effect of renin on blood pressure. It may be postulated that decreased hypertensinase will affect responses to hypertensin less markedly than those to renin. As Gollan, Richardson and Goldblatt have suggested, injected hypertensin acts too quickly to be much affected by this enzyme, while the slow liberation of hypertensin by renin may allow time for destruction by hypertensinase.

Although renin concentration was kept as low as possible to minimize the effect of substrate variation, we cannot eliminate increased hypertensinogen as a factor in our incubations of plasma with renin. In the intact animal increased hypertensinogen is less likely to be of importance since smaller concentrations of renin are ordinarily involved. Our concentration of 0.28 unit of renin per milliliter corresponds to the relatively large total of 140 units or 7 Goldblatt units in the plasma of a 10 Kg. dog with a plasma volume of 5 per cent. It is possible that with moderate doses of renin the concentration of hypertensinogen before nephrectomy may be high enough to give a maximal rate of reaction. In this case increase of hypertensinogen after nephrectomy cannot result in a greater rate of production of hypertensin and hence in a greater pressor response from the same dose of renin.

**SUMMARY**

In 10 dogs, plasma hypertensinase decreased from an average value of 2.1 units per milliliter to 1.4 units per milliliter 48 hours after nephrectomy. These determinations were made in an effort to explain the postnephrectomy increase in the amount of hypertensin resulting from the incubation of plasma with a relatively small concentration of renin.

The mean plasma concentration of hypertensinogen showed an approximately threefold increase following nephrectomy.

The possible roles of decreased hypertensinase and increased hypertensinogen in the increased responses of the nephrectomized animal to renin and hypertensin were discussed.

*According to Walkerlin (personal communication) one Goldblatt unit of renin equals 40 Dexter cat units; one Dexter cat unit equals 0.5 South American unit.
ACKNOWLEDGMENTS

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