The Isolation of Hypertensin from the Circulating Blood of Normal Dogs with Experimental Renal Hypertension by Dialysis in an Artificial Kidney

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Hypertensin has been dialyzed out of the circulating blood of dogs by means of an artificial kidney and a new method has been devised for its recovery from the dialyze. More hypertensin was recovered from the dialyzate obtained from dogs with experimental renal hypertension than from normal animals.

The present paper is a report on experimental procedures designed to determine whether hypertensin is the vasoconstrictor substance responsible for the elevation of blood pressure in dogs with benign experimental renal hypertension. Previous work1 has shown that a pressor material similar in all general respects to hypertensin can be dialyzed out of the circulating blood of dogs by the use of an artificial kidney.2 The pressor material was present in the dialyzates of dogs under the following experimental conditions: (1) 4 dogs with malignant hypertension; (2) 2 out of 9 normal animals; (3) 2 normal dogs in which the artificial kidney was filled with blood from donor dogs which were not nephrectomized; and (4) 3 out of 9 dogs which were injected intravenously with varying amounts of renin. The dialyzates from the dogs were worked up chemically and then injected intravenously into rats to determine the amount and kind of pressor material that was present. The rat was chosen as the test animal because it was found that 0.01 of a Goldblatt unit of hypertensin3 gave a rise of 20 to 30 mm. Hg in the blood pressure of the anesthetized animal. The rise in blood pressure produced in the rats by the injection of the pressor material recovered from the dialyzates was compared to the rise following the administration of 0.01 unit of hypertensin. In both instances it was found that the maximum rise in blood pressure occurred in one minute or less and returned to normal in three minutes or less. The pressor material recovered from the dialyzates was destroyed by incubation with hypertensinase, crystalline trypsin and pepsin. Pharmacologic studies revealed that the pressor material did not produce tachyphylaxis, nor was the pressor effect changed by previous injections of cocaine, atropine or 933 F; however the pressor response was potentiated by a previous injection of tetraethylammonium chloride. The material was destroyed by boiling at a pH of 12 but not at a neutral pH or at a pH of 1. The pressor substance was slowly dialyzable through a cellulose membrane, was water and alcohol soluble and ether insoluble. Thus this pressor material was found to have the general properties of hypertensin.4

The loss of pressor material which occurred during the chemical preparations of the dialyzates in these experiments was so great that no reliable quantitative results could be obtained. The experiments showed only qualitatively that hypertensin was present in the circulating blood of the dogs with malignant hypertension as well as in an occasional normal dog.
It was necessary, therefore, to develop a new method for recovering hypertensin from the dialyzate which would give a more consistent and better yield. The method described in this paper gave a 50 to 80 per cent recovery of hypertensin from the dialyzate.

**EXPERIMENTAL PROCEDURES**

The same experimental procedures were used for the dialyses in these experiments as in our previous work. In brief these methods were as follows. Dogs were used in all experiments and received 15 mg. of morphine sulfate and 0.4 mg. of atropine sulfate 15 to 30 minutes before the experiments were started. The artificial kidney was connected to the femoral artery and femoral vein by means of rubber tubing. The pressure in the femoral artery produced a blood flow of 100 to 200 ml. per minute through the kidney. The experimental animals were dialedyzed for two consecutive 90 minute periods against 500 ml. of circulating dialyzing solution which usually increased by 100 ml. to 200 ml. by ultrafiltration in each 90 minute period. The composition of the dialyzing solution was Na\(^+\) 148, Ca\(^++\) 5, K\(^+\) 3, Mg\(^++\) 3, Cl\(^-\) 126, HCO\(_3\)\(^-\) 24, HPO\(_4\)\(^-\) 2, and lactate 7, mEq. per liter plus 100 mg. of glucose per 100 ml. The pH of the solution was 7.35 to 7.45. At the end of each 90 minute period, the dialyzate was collected, cooled in ice to 3 C. and stored in the deep freeze until chemical processing could be undertaken.

All of the dogs were heparinized, receiving at the beginning of the experiment 4 mg. per Kg. of heparin and throughout the experiment 0.5 mg. per Kg. per hour.

The dialyzing solution was pumped through the kidney by means of a Sigmamotor pump, at a rate of 200 ml. per minute and at a negative pressure of 10 mm. Hg. The dialyzate passed through a copper coil placed in a water bath which was maintained at 39 C. This was found to maintain the temperature of the blood circulating through the kidney at 38.5 C. A needle attached to a mercury manometer was inserted into the rubber tubing which connected the femoral artery with the kidney, so that the approximate mean blood pressure of the animal could be observed throughout the experiment.

Four hundred ml. of blood collected from donor dogs by arterial puncture into a flask which contained 20 mg. of heparin and 20 ml. of normal saline. This blood was used to fill the artificial kidney and the rubber tubing which connected it to the experimental animal. The artificial kidney, the rubber tubing, and the air trap held 250 ml. of blood; 50 ml. were discarded and the remaining 150 ml. of blood were given by slow intravenous drip throughout the early period of the dialysis.

The pressure of the blood in the kidney was raised by adding blood to it with a syringe until it was equal to the arterial pressure of the dog to be dialyzed. By this procedure, there was no loss of blood or fall in the blood pressure when the dialysis was started. All of the donor dogs were bilaterally nephrectomized for at least 18 hours before they were bled and some as long as 96 hours. Nephrectomized donor dogs were used in order to make sure that the hypertensin recovered in the dialyzate came from the animals that were being dialyzed, and not from the donor dogs.

The following methods were used in preparing the dialyzates for intravenous injections into rats. The dialyzate was removed from the deep freeze, thawed in warm water and processed in 300 ml. quantities. Each 300 ml. was subjected to the following procedures. The pH of the solution was immediately adjusted to 3±0.1 by the addition of 8 to 10 ml. of 1 N hydrochloric acid and then was vacuum concentrated to 10 ml. in a one liter round bottom flask at temperatures below 20 C. Twenty-five ml. of redistilled n-butanol was added, the flask was stoppered, shaken for five minutes, transferred to a 50 ml. centrifuge tube and then centrifuged for two to three minutes in order to break the emulsion. The butanol layer was transferred to a 250 ml. Erlenmeyer flask. The one liter flask used for evaporation was washed with 25 ml. more of butanol decanted into the centrifuge tube containing the original water layer, stoppered, shaken for five minutes, centrifuged and the second butanol layer was combined with the first one in the Erlenmeyer flask. The hypertensin was now dissolved in the butanol while most of the salts remained in the water. The hypertensin was removed from the butanol by adsorption on to 5 Gm. of alumina which was added to the Erlenmeyer flask and agitated for five minutes.

The alumina\(^4\) (activated 80 to 200 mesh Fisher Scientific Co.) was prepared in the following fashion: 600 Gm. of alumina were shaken intermittently for one hour with 3 liters of concentrated hydrochloric acid diluted 1:5 with distilled water. The hydrochloric acid was filtered off, the alumina was washed thoroughly with distilled water (about 30 gallons) until the wash water, when treated with silver nitrate, gave only a faint opalescence. The alumina was then air dried for 48 hours.

The butanol with the suspended alumina was poured into a 50 ml. medium porosity sintered glass funnel, fitted for vacuum filtration. The butanol was filtered off, discarded and 40 ml. of 90 per cent ethanol were added to the alumina, stirred for one minute, and then filtered off. The alcohol wash was repeated once more and the ethanol washes were discarded. The hypertensin was removed from the alumina by washing it twice with two 40 ml. quantities of distilled water which were stirred each time with the alumina for five minutes. The water with
the dissolved hypertensin was then filtered off the alumina and poured into a beaker containing 4 Gm. of Amberlite (IR-4-B Rohm & Haas). The Amberlite was prepared for use in the following manner: 200 Gm. of Amberlite were washed thoroughly with running distilled water until the wash water showed no opalescence with phosphotungstic acid. The Amberlite and water containing the dissolved hypertensin were stirred until the pH had risen to 6.1 ± 0.1. The resin was filtered off immediately and discarded. The filtrate containing the hypertensin was concentrated under a vacuum in a 500 ml. round bottom flask to approximately 3 ml., and then transferred to a 15 ml. centrifuge tube with the aid of small washes of distilled water. A drop of phenol red indicator was added and the pH adjusted to approximately 7.0 by the addition of either 0.1 N hydrochloric acid or sodium hydroxide solutions. The solution was centrifuged at high speeds to remove small amounts of insoluble materials. The clear supernatant fluid was transferred to a 10 ml. ampoule, shell frozen and then lyophilized. Following lyophilization, the ampoule was sealed and stored at room temperatures until it could be assayed for its content of hypertensin. At the time of the assay, the material was dissolved in either 0.5 or 1.0 ml. of distilled water.

Assays of the dialyzates were performed on mature rats anesthetized with Sodium Amytal, 9 mg. per 100 Gm. of body weight injected intraperitoneally. Atropine, 0.4 mg., was administered intraperitoneally at the same time the Amytal was given. Thirty minutes after the administration of the anesthetic, both vagus nerves were cut and the trachea was cannulated. A needle was inserted and tied into the left jugular vein; this was used for intravenous injection of the material that was to be assayed. The right carotid artery was cannulated and connected with a capillary mercury manometer equipped with a pen for ink writing on a kymograph.

All of the tests on the dialyzates were done with small volumes ranging between 0.25 and 0.50 ml. The rises in the rats' blood pressure were compared with those obtained by the injection of 0.01 unit of hypertensin prepared from standard lyophilized powder. One unit of this standard gave an average rise in blood pressure of 32 mm. Hg in 12 nembutalized rabbits and an average 30 mm. rise in 7 unanesthetized dogs. This unit was approximately equal to the Goldblatt unit.

By the methods that were used in the preparation of the dialyzates, the pressor material was found to be slowly dialyzable through a cellophane membrane. It was not destroyed by freezing or lyophilizing. The pressor material which was present in minute quantities in the dialyzates could be extracted into butanol from the acidified concentrated salt solution which was essentially what the dialyzates were at this stage in their chemical processing. The pressor material was adsorbable onto alumina from the butanol or 90 per cent ethanol and could be eluted from it by the addition of water. These properties are characteristic of hypertensin.

Ten dialyzates from hypertensive dogs that contained hypertensin were tested for destruction of the pressor effect of this material by incubation with hypertensinase and trypsin. Several control tests were performed using heat-inactivated trypsin and hypertensinase. The pressor effects of the dialyzates were destroyed by incubation with trypsin and hypertensinase.

A large quantity of a material such as hypertensin must be available if a complete study of its chemical components and biologic characteristics are to be undertaken. No such comprehensive study could be done on the pressor material obtained in the dialyzates from the circulating blood of the dogs, because it was present in such small amounts. The number of chemical and biologic tests for identifying it had to be limited to the ones that gave the most information as to its nature. The results of the chemical and biologic tests given above show that the pressor material obtained had all of the general properties and characteristics of hypertensin. An analysis of a pooled sample of 22 different preparations gave the following average composition for each 300 ml. of chemically prepared dialyzed: sodium, 0.164 mEq.; potassium, 0.0079 mEq.; chloride, 0.177 mEq.; glucose, 1.0 mg.; urea nitrogen, 0.33 mg.; total nitrogen, 1.23 mg.; total solids, 24.5 mg.

One control preparation was run for each experiment in order to determine the amount of hypertensin that was lost during the processing of the dialyze. The control preparation consisted of 300 ml. of dialyzed, which contained no pressor material, obtained from normal dogs to which 0.02 to 1.0 unit of hypertensin was added, and worked up in the same manner as the dialyze from the experimental animals. Forty-nine such control preparations were done and the recovery of hypertensin ranged from 50 to 80 per cent with an average yield of 60 per cent.

Experiment 1. Eleven dogs, made hypertensive by means of moderate constriction of one or both renal arteries by a Goldblatt clamp,6 were subjected to dialysis of their blood in the artificial kidney. Nine normal dogs were also subjected to dialysis in the same manner to determine whether there was a significant difference in the amount of hypertensin recovered from the dialyzates of the hypertensive dogs compared with that recovered from the normal animals. (See tables 1 and 2.)

Experiment 2. Six bilaterally nephrectomized dogs were subjected to dialysis to determine whether hypertensin was present in the circulating blood of animals with both kidneys removed. A pressor material was found in the dialyze of only 1 of the dogs and it was not present in sufficient amounts to determine whether or not it was hypertensin.
Table 1.—Dogs with Benign Experimental Renal Hypertension

| Dog Number | Pressure | Operations | Number of days dialyzed | Number of days hypertensin per liter of dialyze
<table>
<thead>
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<tbody>
<tr>
<td>42</td>
<td>140</td>
<td>LK</td>
<td>4</td>
<td>1st flask - 0.20 U/L 2nd flask - 0.08 U/L</td>
</tr>
<tr>
<td>43</td>
<td>150</td>
<td>LK</td>
<td>6</td>
<td>1st flask - 0.21 U/L 2nd flask - 0.09 U/L</td>
</tr>
<tr>
<td>32</td>
<td>200</td>
<td>LK &amp; RK</td>
<td>7</td>
<td>1st flask - 0.33 U/L 2nd flask - 0.02 U/L</td>
</tr>
<tr>
<td>28</td>
<td>165</td>
<td>LK &amp; RK</td>
<td>10</td>
<td>1st flask - 0.20 U/L 2nd flask - 0.08 U/L</td>
</tr>
<tr>
<td>29</td>
<td>175</td>
<td>LK</td>
<td>15</td>
<td>1st flask - 0.08 U/L 2nd flask - 0.08 U/L</td>
</tr>
</tbody>
</table>

Table 2.—Normal Dogs

| Dog Number | Units of hypertensin per liter of dialyze
<table>
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<tr>
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<tbody>
<tr>
<td></td>
<td>1st 90 min. per. of dialysis 2nd 90 min. per. of dialysis</td>
</tr>
<tr>
<td>34</td>
<td>1st flask - trace 2nd flask - 0.04 U/L</td>
</tr>
<tr>
<td>37</td>
<td>1st flask - trace 2nd flask - 0.04 U/L</td>
</tr>
<tr>
<td>38</td>
<td>1st flask - 0.04 U/L 2nd flask - trace</td>
</tr>
<tr>
<td>39</td>
<td>1st flask - 0.04 U/L 2nd flask - 0.04 U/L</td>
</tr>
<tr>
<td>43</td>
<td>1st flask - 0.06 U/L 2nd flask - 0.03 U/L</td>
</tr>
<tr>
<td>44</td>
<td>1st flask - 0.05 U/L 2nd flask - 0.06 U/L</td>
</tr>
<tr>
<td>45</td>
<td>1st flask - trace 2nd flask - trace 2nd flask - broken</td>
</tr>
<tr>
<td>42</td>
<td>1st flask - trace 2nd flask - 0</td>
</tr>
<tr>
<td>32</td>
<td>1st flask - trace 2nd flask - trace</td>
</tr>
</tbody>
</table>

Experiment 3. Six bilaterally nephrectomized dogs were dialyzed for two consecutive 90-minute periods, during which they were infused with hypertensin. The hypertensin was administered intravenously at a constant rate by means of a syringe which was driven at a constant speed. Four dogs received 0.43 unit of hypertensin per minute and 2 dogs received 1 unit of hypertensin per minute. The first group of animals received 36 units of hypertensin during each 90 minute period and the second group 90 units. The infusion of hypertensin elevated the blood pressure 20 to 30 mm. Hg in the first group of animals, and 25 to 35 mm. Hg in the second.
This group of experiments was performed for two reasons. (1) In order to determine if the recovery of hypertensin from the dialyzates would be in the same order of magnitude as was the hypertensin which was recovered from the dialyzates of the dogs with experimental renal hypertension. (2) To determine whether the same amount of hypertensin was recovered in the second 90 minute period of dialysis as in the first (see table 3).

**DISCUSSION**

Hypertensin has been dialyzed out of the blood of normal dogs and dogs with benign experimental renal hypertension by means of an artificial kidney. It was recoverable in significantly larger amounts from the animals in the early phases of experimental hypertension than in those with long continued elevation of their blood pressures or in the normal dogs. (Dog 31 had renal insufficiency and died of malignant hypertension.)

The recovery of hypertensin from bilaterally nephrectomized dogs infused with this substance shows that the methods used for its recovery in these experiments would have demonstrated it, if it were present in sufficient amounts in the circulating blood of the intact dog. Tables 1 and 2 show that there was a marked drop in the amount of hypertensin recovered during the second 90 minute period of dialysis in the hypertensive and in the normal animals. There was no decrease, however, in the amount of hypertensin recovered in the second 90 minute period in the animals that received a constant intravenous infusion of this material. This finding ruled out the possibility that the cellophane membrane became progressively less permeable to the hypertensin molecule, and it means that there was less hypertensin circulating in the dogs' blood during the latter part of the experiments. It is true that the blood pressures of animals with experimental hypertension and hypertensive human beings tend to fall somewhat when they are put at complete rest. It may be inferred from this fact that less hypertensin is formed at such times and this could be an explanation for the decrease in the amount of hypertensin recovered from the dogs during the second 90 minutes of dialysis.

From the experimental data it is impossible to conclude with certainty that hypertensin is the vasoconstrictor material responsible for the elevation of blood pressure in benign experimental renal hypertension. The findings, however, do suggest this possibility, since at least in the majority of hypertensive dogs more hypertensin was recovered in the dialyzates from both the first and second periods than was found in the normal animals. It is also of interest, although it may be only a coincidence, that the amount of hypertensin recovered from the animals with early experimental renal hypertension and those which received a constant infusion of this material was of about the same magnitude.

The possibility that the experimental procedures were in themselves sufficient to cause the kidneys to form renin and thus liberate hypertensin into the blood could not be ruled out, for the application of the artificial kidney to the dogs created a large arteriovenous shunt. There was not, however, a fall in blood pressure when the dialyses were first started, so that a drop in the blood pressure could not be incriminated as the stimulant for the production of renin and the liberation of hypertensin. The abnormal position of the dogs during the dialyses, the injection of morphine and atropine, and the transfusion of 400 ml. of blood from a uremic donor dog could possibly have stimulated the production of renin in the kidneys of the dogs that were dialyzed. If these assumptions were true, they still do not explain why more hypertensin was obtained from the hypertensive than from normal dogs, when they were subjected to the same experimental procedures.

If renin were present in the circulating blood, its action on its substrate, hypertensinogen, could be sustained or even accelerated during its passage through the artificial kidney because of its extravascular position. For this reason, it cannot be concluded with finality that the methods employed are a test for hypertensin alone, but may be a test for renin as well. It would be unwise, therefore, to make a strict comparison of the amount of hypertensin recovered in the infusion experiments with the amounts recovered from the hypertensive or normal dogs.

A simpler method for isolating hypertensin from the blood than by dialysis in an artificial
kidney must be developed before there can be a definite conclusion reached as to whether hypertensin is the chemical mediator responsible for the elevation of the blood pressure in experimental renal hypertension. The results obtained in these experiments suggest that hypertensin may play an important role in initiating and maintaining the elevation of blood pressure at least in the initial phases of experimental renal hypertension. Indeed hypertensin was recovered from the dialyzates of all of the hypertensive dogs even those with chronic benign experimental renal hypertension, although the amount is much reduced and tends to overlap that recovered from an occasional normal dog. These findings are in agreement with those of Goldblatt.7

**Summary**

1. Hypertensin has been dialyzed out of the circulating blood of normal dogs as well as dogs with experimental renal hypertension.
2. Hypertensin was obtained in significantly larger amounts from the animals in the early phases of experimental renal hypertension than in those with long continued elevation of their blood pressure or in normal dogs.
3. A new method for the recovery of hypertensin from the dialyzate, which yielded a 50–80 per cent recovery of this material, has been described.

**REFERENCES**

The Isolation of Hypertensin from the Circulating Blood of Normal Dogs with Experimental Renal Hypertension by Dialysis in an Artificial Kidney

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