On Vascular Permeability Factor from the Kidney Cortex

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SUMMARY

(1) The vascular permeability factor is present in the renal cortex. This factor has no pressor activity and is quite different from renin.

(2) The vascular permeability factor has the characteristics of a protein. Immunoelectrophoretic study reveals a single precipitation line.

(3) Administration of the vascular permeability factor to mice causes cerebral hemorrhage.

(4) Rabbits subjected to renal ischemia develop cerebral hemorrhages but are provided protection against it through lanolin feeding.

(5) Some evidence is presented indicating that the renal vascular permeability factor plays an important role in the development of vascular lesions.

Additional Indexing Words:
Vascular lesion Renal ischemia Hypertension Gel-filtration Hemagglutination-inhibition reaction Atheroma Aortic arteriosclerosis Cerebral hemorrhage Immunofluorescent technique Antiserum against microsome fractions of renal cortex

In recent years experimental and clinical evidences have accumulated implying that hypertension is a common antecedent of vascular lesion including arteriosclerosis. But the origin and nature of the vascular lesions and their relation to the high blood pressure are not clearly established.

In previous experimental studies it was shown that several severe renal ischemia in experimental animals brought about widespread vascular lesions and hypertension. Under this circumstance a release into the blood or lymph stream of several substances escaping from ischemic kidney cells will occur. Renin was at first taken into account as one of these substances and several attempts have been made to reproduce renal hypertensive vascular disease by administration of renin or angiotensin. But somewhat conflicting results have been reported.

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Received for publication May 27, 1968.

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In our previous papers\textsuperscript{1)–5)} it was shown that increased vascular permeability played an important role in the development of vascular lesions. On the other hand, Giese\textsuperscript{6)} reported that severe renal ischemia in rats gave rise to increased vascular permeability as evidenced by manifestations such as tissue edema, serious effusions and deposits of serum proteins in arterial and arteriolar walls.

The present study is aimed at showing whether a permeability factor is present in the kidney. If this is the case, there is a strong possibility that an outpouring of this permeability factor into the blood stream from the ischemic kidneys is the cause of the vascular lesions.

\textbf{Materials and Methods}

1. Production of hypertension

White male rabbits weighing 2.0 to 3.0 Kg. were used. One week after applying a silver clamp of 0.65 mm. in diameter to the right renal artery, the left renal artery was constricted by a clamp of 1.1 mm. in diameter.

2. Measurement of blood pressure

Arterial pressures in these animals were measured on the central artery of the ear.

3. Preparation of a vascular permeability factor from the kidney cortex

Kidneys of rabbits, rats and pigs were extensively perfused through a polyethylene tube inserted in the renal artery. The perfusion fluid was 10L. of saline added heparin. After the last traces of blood had been washed out, the medullar layer was removed and the cortical layer cut into small pieces which were repeatedly washed with cold saline to eliminate the hemoglobin. The washed tissue was weighed and suspended in 2 volumes of the distilled water and then homogenized. The homogenates were kept over night in a cold room, and then centrifuged at 40,000 g. for 30 min. and the supernatant recentrifuged at 100,000 g. for 60 min.

4. Gel filtration

Gel filtration columns (25 cm. to 3.0 cm. diam.) of Sephadex G 100 were packed in the cold with the swollen gels. Effluent was collected in 5 ml. fractions by using a fraction collector (Shibata Chemical Company Ltd., Tokyo, FTV-20). All columns were run at 15\degree C and flow rates were 30 ml./hour.

5. Electrophoresis

Electrophoresis was done on cellulose acetate strips using an electrophoresis cell filled with veronal buffer solution (pH 8.6, ionic strength 0.1). One to 3 ml. of the concentrated sample was applied on the strips and a current of 3 mA. per strips was passed through the strips for 30 min. The strips were then stained with Ponceau 3 R.

6. Preparation of chicken antisera against subfraction 45 of rabbit’s kidney cortex

The starting material was the homogenate of the washed cortical layer of rabbits. And fractionation of subfraction 45 was performed according to modified Hogeboom-Schneider’s procedure.\textsuperscript{7)–10)} Chicken antisera against subfraction 45 were prepared by subcutaneous injections of the fraction in Freund’s complete adjuvant.
7. Observation of the vascular permeability factor localization in kidney

The localization of the vascular permeability factor in kidneys was investigated by means of immunofluorescent technique. Rabbit antisera against subfraction 45 of human kidney cortex were prepared by the same method as mentioned above. A concentrated globulin fraction of the antiserum was obtained using the method of Griffin. A concentrated globulin fraction of the antiserum was obtained using the method of Griffin. It was conjugated with fluorescein isothiocyanate. A human kidney obtained at an autopsy was quickly frozen in n-Hexan. Frozen sections, 4 to 6μ thick, were cut in a cryostat. Sections were transferred to a glass slide, washed in phosphate buffered saline and stained with the fluorescent antibody. A fluorescein microscopy was done.

8. Measurement of vascular permeability

0.05 ml. of the test solution was injected intracutaneously into the depilated skin of the flank of a rabbit or rat. Thirty min. later, the animal received an intravenous injection of Evans blue, 0.07 ml./100 Gm. body weight.

9. Hemagglutination-inhibition reaction

The procedure for preparation of formalinized human red cells and of tannic acid cells and that for sensitization of the tanned cells are shown in Fig. 1 and 2.

One volume of 3% solution of formalin
+

One volume of 8% suspension of human red cells
(washed 5 times with saline)
↓ incubated at 37°C for 20 hours

The formalin was removed by washing with distilled water
↓

The formalinized cells were kept at 4°C in 10% suspension of saline with 1/10,000 Merthiolate

Fig. 1. Preparation of formalinized erythrocytes.

One volume of 3.3% suspension of formalinized tanned cells in pH 6.4 phosphate buffered saline
+

One volume of rabbit kidney cortex (human kidney cortex)
↓ incubated at 37°C for 1 hour

The cells were washed two times with saline, and finally stored in 10% saline containing 1/10,000 Merthiolate

Fig. 2. Sensitization of formalinized tanned cells.

Antisera were diluted with physiologic saline to 1:500. 0.1ml. of 1:50 dilution of serum to be tested was added to a series of 10 tubes containing 0.1 ml. of the diluted antisera. The mixture was incubated at 37°C for 30 min. and then 3 percent suspension of sensitized cells was added to the mixture. The reaction was read after 24 hours.

RESULTS

1. Hypertension, arteriosclerosis and cerebral hemorrhage induced by constriction of the main renal arteries
Table I. Relationship of Blood Pressure, Arteriosclerosis and Cerebral

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2   3   4   5   6   7</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>Before death</td>
</tr>
<tr>
<td></td>
<td>190 113 127 124 102 86 192</td>
</tr>
<tr>
<td></td>
<td>Mean value during the</td>
</tr>
<tr>
<td></td>
<td>133 83 120 138 117 99 185</td>
</tr>
<tr>
<td></td>
<td>Average value of group</td>
</tr>
<tr>
<td></td>
<td>125.1</td>
</tr>
<tr>
<td>* Degree of aortic</td>
<td>+</td>
</tr>
<tr>
<td>arteriosclerosis</td>
<td>+</td>
</tr>
<tr>
<td>** Degree of cerebral</td>
<td>-</td>
</tr>
<tr>
<td>hemorrhage</td>
<td>-</td>
</tr>
<tr>
<td>Survival time after</td>
<td>218 209 116 200 261 563 442</td>
</tr>
<tr>
<td>constriction of renal</td>
<td></td>
</tr>
<tr>
<td>arteries</td>
<td></td>
</tr>
<tr>
<td>Average survival time</td>
<td>288.3</td>
</tr>
<tr>
<td>of group</td>
<td></td>
</tr>
</tbody>
</table>

* ~ + : Degree of aortic arteriosclerosis without atheromatous lesion such as lipid deposits thickening and calcium deposits.
( - ~ + : Degree of aortic arteriosclerosis.)
** ~ : Degree of microscopic findings of cerebral hemorrhage.
- ~ : Degree of macroscopic findings of cerebral hemorrhage.
( - ~ ( ) : Case of cerebral hemorrhage with bleeding of other organs such as stomach,

Twenty-five rabbits were subjected to constriction of the main renal arteries and divided into 2 groups. The control A group received a semisynthetic diet and group B a semisynthetic diet containing 10% lanolin.

The results are summarized in Table I.

Blood pressure: Seventeen of the 18 control rabbits and all 7 of group B died during the experimental period of 904 days. The average blood pressure of group A was 125 mm. of mercury and that of group B was 101 mm. of mercury.

The aortas: In 9 of the 17 animals in group A and in all 7 of group B, arteriosclerotic lesions were observed in the aorta. Aortic lesions of group A were completely different from those of group B by a lack of atheromatous lesions. In group A the earliest change was degeneration of muscular tissue of the media, followed by swelling of the media and contorsion and disappearance of elastic elements (Fig. 3). In advanced cases of arteriosclerosis, there were intimal thickening, medial calcification and necrosis leading to aneurysm (Fig. 4). In the lanolin fed group, typical atheromatous lesions were observed. Moreover, there was no medial necrosis as observed in the control group.

Cerebral hemorrhages: Brains were examined macroscopically and microscopically for hemorrhages and graded on a 0 to 3 scale.

In group A cerebral hemorrhages were demonstrable in all 17 autopsies whereas only 3 of 7 animals in group B had cerebral hemorrhages. In group
Hemorrhage in the GB+Lanolin (Group B) and GB Group (Group A)

| Group A | 7  | 10 | 14 | 15 | 17 | 19 | 23 | 24 | 25 | 59 | 60 | 67 | 68 | 69 | 70 | 72 | 73 |
|---------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
|         | 97 | 158| 122| 142| 127| 151| 126| 87 | 102| 105| 127| 104| 122| 110| 75 | 140| 61 |
|         | 109| 149| 103| 117| 119| 112| 130| 94 | 104| 104| 107| 104| 106| 109| 110| 83 | 59 |
|         | 100.9 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| +       | +  | #  | -  | -  | $  | +  | -  | +  | #  | -  | $  | -  | +  | -  | +  | -  | -  |
| (−)    | (−) | (+) |−  | (++)|−  | (−) |−  | (+) |(++)|−  | (−) |−  | (−) |−  | (−) |−  | (−) |
| 182    | 150| 183| 87 | 114| 35 | 594| 615| 284| 277| 177| 73 | 499| 57 | 112| 539| 73 |
|         | 238.3 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

and infiltration. These lesions are divided into 4 classes depending on medial necrosis, intimal intestine etc.

A, cerebral hemorrhages were widespread and larger than in group B.

The coronary arteries: Histologically, elastofibrotic lesions were observed in group A, while in group B lesions were typically atheromatous. In group A, smaller arteries measuring 1 to 100 μ. were more affected than in group B.
Hemorrhages in other organs: In 12 of the 17 rabbits in group A hemorrhages of various sizes were observed in the myocardium, gastric submucosa, intestinal submucosa, mesenterium, diaphragm, greater omentum, anterior chamber, lung and bladder, but none was seen in the animals of the lanolin fed group.

Out of another series of 28 rabbits rendered hypertensive from renal ischemia, 27 showed hemorrhages in the gastric submucosa. These animals were killed 44 days after the operation.

2. Localization of permeability factor in the kidneys

The renal cortex was found to contain a permeability factor which was hardly detected in the medulla. The content of the permeability factor in the clamped kidneys was distinctly higher than that in non-operated kidneys, but the content in the initially operated kidneys was gradually decreased.

Fig. 5. Chromatography on Sephadex G 100 of supernatant fraction separated from extract of rabbit kidney cortex.

Fig. 6. Chromatography on Sephadex G 100 of sediment fraction separated from extract of rabbit kidney cortex.
Fig. 5 and 6 illustrates the results of gel filtration of the 100,000 g. rabbit kidney cortex supernatant and sediment fraction. Gel filtration of the supernatant revealed 2 peaks, while that of the sediment fraction had a single peak. The former of the peaks possessed potent vascular permeability activity, but had no effect on blood pressure and respiration. This is the case for human, rat and pig kidney cortices. Vascular permeability activity was separable from pressor activity by this procedure.

From the observation of the vascular permeability factor localization in the human kidney by means of immunofluorescent technique, we obtained specific organ staining of the human kidney cortex, especially of Bowman's capsule and their surrounding area.

The 100,000 g. human kidney cortex supernatant was subjected to the following examination.

3. Ultraviolet light absorption spectra of the kidney extract

The human kidney cortex extract contained in Visking 27/32 in. tubing

![Fig. 7. The ultraviolet absorption spectrum of the vascular permeability factor from kidney cortex.](image)

![Fig. 8. Electrophoretic pattern of vascular permeability factor isolated from human kidney cortex.](image)
was dialysed at 6°C against distilled water. Ultraviolet absorption spectra of euglobulin and non-euglobulin fractions were shown in Fig. 7.

4. Paper electrophoresis of human kidney cortex extract

With Ponceau 3 stain, several fractions were revealed. It should be noted that there were 2 peculiar fractions which were located in the prealbumin and post-gamma-globulin region (Fig. 8).

5. Permeability activity of the renal cortex extract

Intradermal administration of the renal cortex extract to Evans blue injected rats produced a strong leakage of the dye from the vascular component.

6. Cerebral hemorrhage caused by the intravenous injection of the renal cortex extract

Mice were injected intravenously with 0.2 ml. of the fraction obtained by Sephadex G-100 gel filtration which has permeability activity, but no pressor activity. They were killed under ether anesthesia. Five hours later a microscopic examination of the brain revealed hemorrhages, but none was seen in the mice which had received an intravenous injection of physiological saline solution.

7. Inactivation of renal cortex permeability factor

Heating the human renal cortex extract to 90°C for 5 min. resulted in a complete loss of permeability activity. The permeability activity was also easily destroyed by incubation with antiserum against microsome fractions of the renal cortex.

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Fig. 9. Vascular permeability.

GL-C: Extract of rabbit kidney cortex with renal artery constriction.
N-C: Extract of normal control rabbit kidney cortex.
N-M: Extract of normal control rabbit kidney medulla.
8. Demonstration of a vascular permeability factor in the sera of hypertensive rabbits

Blood was withdrawn from rabbits before and 4 days after the constriction of main renal arteries. The sera were injected intradermally into rats which had received an intravenous injection of Evans blue. The serum of hypertensive rabbits produced an intense blueing response, but the serum prior to the operation produced only some blueing.

It is conceivable that a permeability activity of renal origin is responsible for the increase in the permeability activity of the serum after the constriction of the main renal arteries (Fig. 9).

9. Effect of fat loading on vascular permeability

Rats were fed atherogenic diets for 2 weeks. Loose connective tissues of the flanks of rats were exposed. Hyperlipemic rats received an intravenous injection of Evans blue, 0.07 ml./100 Gm. body weight, after 10 min. followed by intravenous injection of 0.2 ml. of the renal cortex extract. As compared to untreated rats, vascular leakage was significantly reduced in the hyperlipemic animals.

Fig. 10. Immunoelectrophoretic pattern.
Vascular permeability factor in human kidney cortex was placed in the upper well, normal human serum in the lower well, and antihuman serum rabbit serum in the central trough.

Fig. 11. Immunoelectrophoretic pattern.
Vascular permeability factor in human kidney cortex was placed in the upper trough, normal human serum in the lower trough, and anti-human kidney cortex vascular permeability factor rabbit serum in the central well.
10. Immunoelectrophoresis

Agar gel immunoelectrophoresis on glass plates (7.5 by 2.5 cm.) was performed with 1.0% special agar Noble (Difco) in veronal buffer (pH 8.6, ionic strength 0.025). After the renal cortex extract to be studied was electrophoresed, troughs were filled with antisera (Behringwerke). The patterns were allowed to develop for 20 hours at 4°C. The renal cortex extracts revealed no precipitin line with antiserum against human sera (Fig. 10) and a single line with antiserum against kidney microsome (Fig. 11).

11. Hemagglutination-inhibition reaction

Hemagglutination-inhibition tests were used to determine whether the renal vascular permeability factor is present in the blood after the operation or not. Sera of hypertensive rabbits gave an inhibition of the hemagglutination reaction using cells sensitized with the renal cortex extract, while the sera prior to the operation gave a hemagglutination reaction indicating only a small amount of the renal permeability factor (Fig. 12).

Fig. 12. Hemagglutination inhibition reaction.

V: before constriction of renal arteries.
N: after constriction of renal arteries.

DISCUSSION

Hypertension produced by renal artery constriction was developed by Goldblatt in 1934[12] and many investigators have tried, by a great variety of methods, to induce the development of hypertension in animals.[13]–[18] Cerebrovascular lesions were reported in these hypertensive animals, but their incidence was very low. Furthermore, there remains considerable difference of opinion concerning factors responsible for their production. Almost all of the animals with renal artery constriction by our method developed cerebrovascular lesions. This was attributable to shortening of the arms of the clip.
The clip with long arms, as used in the original report of Goldblatt, was displaced and was not able to constrict renal arteries accurately. On the other hand, displacement of our clips was not observed and expected constrictions were obtained.

Another characteristic lesion of our animals was severe aortic lesion which has not been reported in hypertensive rabbits. This lesion consisted mainly of medial necrosis and intimal thickening.

Koletsky et al. noted some increase of vascular permeability in experimental renal hypertension. Our previous work indicated that increased vascular permeability played an important role in the development of arteriosclerotic lesions. On the other hand, Giese reported that severe renal ischemia in rats gave rise to increased vascular permeability as evidenced by manifestations such as tissue edema, serous effusions and deposits of serum proteins in arterial and arteriolar walls. We reached the working hypothesis that arterial lesion in our animals, including cerebral hemorrhages and aortic lesions, is due to a vascular permeability factor released from ischemic kidney.

In agreement with previous workers, a permeability factor was identified in renal cortex, but absent from medulla. Several studies suggested that the vascular permeability factor present in the cortex of the kidneys was renin or some substances with similar properties. Some fractions obtained by gel filtration on Sephadex G-100 of renal cortex extract were found to have permeability activity, but no pressor activity. Furthermore, the localization of fluorescent antibody to permeability factor was quite different from that of fluorescent antibody to renin. From these data it is concluded that our permeability factor is quite different from renin.

Asscher et al. noted that the renal cortex of rats contained a heat labile substance which increased vascular permeability to plasma proteins in nephrectomized and renal hypertensive rats. The present observations establish that the vascular permeability factor of renal cortex origin is also heat labile, and easily inactivated by incubation with antiserum against microsome fractions of the renal cortex, forming insoluble antigen-antibody complexes. Moreover, in immunoelectrophoresis, no cross-reactions were observed between the permeability factor and serum protein. On these grounds, it is postulated that the permeability factor had the characteristics of a protein and has organ-specific antigens.

The biological assay for permeability activity as well as hemagglutination inhibition reaction using red cells conjugated with the renal cortex extract revealed an increase in the permeability activity of rabbit serum after the constriction of renal arteries. Furthermore, the present results are of particular interest in understanding the pathogenesis of human vascular lesions.
REFERENCES