Increased Permeability of Arteriolar Wall Caused by Renal Extract in Nephrectomized Rats

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SUMMARY

Lysosomal fraction of high renin activity, obtained from renal cortex of normal rats by step-wise centrifugation method, phenylephrine and angiotensin II were injected into bilaterally nephrectomized rats. Blood pressure was raised 20 mmHg for 60 min by the pressor agents. Ferritin was used to trace pathways of plasma protein insudation in the arterial wall. Animals infused with saline served as a control. Arterioles in the intestinal submucosa was observed by light and electron microscope. The arterial endothelium and the interendothelial cell junctions appeared to be intact in all the experimental animals. Ferritin was present in pinocytotic vesicles in the endothelial cells and in the subendothelial spaces, but never in the intercellular junctions in each of the experimental groups. Ferritin concentration in the subendothelial spaces, however, was the highest in lysosomal fraction group. Morphometric analysis concerning the vesicular transport, which appeared to be the main pathway of ferritin insudation, was performed in the arterial endothelium. Frequencies of the pinocytotic vesicles in the experimental groups resembled each other. Percentage of the pinocytotic vesicles labeled with ferritin, however, was significantly higher in lysosomal fraction group than that in the other groups. The results suggested that substance(s) contained in lysosomal fraction may exert direct action on the arterial endothelium and accelerate the labeling rate of pinocytotic vesicles with macromolecules, resulting in insudation of the plasma protein into subendothelial spaces of the arterioles.

Additional Indexing Words: Renin Angiotensin II Phenylephrine Vesicular transport

Renal extracts cause an increase in vascular permeability to plasma protein, a prolonged rise of blood pressure and arterial necrosis similar to the lesions of malignant hypertension, when injected into bilaterally neph-

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rectomized animals. Many investigations, on the other hand, have suggested that insudation of plasma proteins into arterial wall plays a very important role in development of arterial necrosis in experimental renal hypertension. It is controversial, however, whether the increase in vascular permeability depends on the rise of blood pressure per se or is due to the direct effect of renal extract(s) on the vessel wall. To solve the problem in this regard, it is necessary to compare the vascular permeability in animals receiving renal extract with that in animals injected with the other pressor agents, in the same condition in respect of duration and height of blood pressure rise.

In acute severe hypertension induced by renal extract or angiotensin it is reported that macromolecules pass through pathologic gaps of the endothelium and insudate to subendothelial space of the arteries. In physiological condition, on the other hand, protein molecules enter arterial subendothelium via 2 selective pathways; intercellular clefts and vesicular transport. It is also suggested that transport of plasma proteins through the selective pathways may be accelerated in chronic hypertensive condition. However, there are few reports concerning an effect of the renal extract and angiotensin on the physiological pathways.

The aim of the present study was to evaluate the differences in the effect of pressor agents such as phenylephrine, angiotensin and renal extract on the vascular permeability of arterioles. Mean arterial pressure of rats was raised about 20 mmHg for 60 min by administration of the pressor agents. Vesicular transport in the arterial endothelium was analysed by means of morphometric technique, and intercellular passage through the cleft was studied by utilizing ferritin as a tracer.

MATERIALS AND METHODS

Female Wistar-King rats weighing approximately 200 Gm were used in the experiments. All the animals were anesthetized with intraperitoneal injection of amobarbital sodium and the experiment was made under the anesthesia. In order to exclude any participation of the renal renin-system and sustained pressor response to angiotensin and renal extract, both kidneys were removed through loin incision 1 hour before administration of the pressor agents. The right carotid artery and femoral vein were cannulated. Mean arterial pressure was recorded from the carotid artery using a strain-gauge pressure transducer. Synthetic angiotensin II amide (Hypertensin, Ciba) dissolved in normal saline and phenylephrine were infused in variable doses into femoral vein using a multi-speed infusion pump. As renal extract with high renin content, lysosomal fraction obtained from renal cortex of the normal rats by stepwise centrifugation method was used. The lysosomal fraction dissolved in saline, whose doses were expressed by protein concentra-
tion, was intraperitoneally injected. The 3 pressor substances in variable doses caused sustained increase in mean arterial pressure for 60 min of experimental period, and their dose-response curves were determined. Doses of each pressor agent raising 20 mmHg of mean arterial pressure was 3.0 ng/Kg/min of phenylephrine, 0.08 ng/Kg/min of angiotensin II, and 1.0 mg/ml of lysosomal fraction. Five min prior to the administration of pressor agents, 10% ferritin solution (NBC, Cleveland, Ohio, USA) was injected into femoral vein in a doses of 0.55 ml/100 Gm body weight as a tracer. The injection of ferritin caused no changes in pressor response to each agent. Total volume of the parenteral route was restricted to 0.7 ml/100 Gm body weight, estimated to be approximately 10% of blood volume of a rat. Experimental groups were divided into 3; phenylephrine, angiotensin II, and lysosomal fraction group. Five animals were used in each experimental group. Five rats infused with 0.15 ml/100 Gm body weight of physiological saline and injected with 0.55 ml/100 Gm body weight of 10% ferritin solution were also used as a control group. On 60 min after the starting of the administration of pressor agents and 65 min after ferritin injection, 20 ml of a 3% formaldehyde–3% glutaraldehyde mixture in 0.1 M cacodylate buffer (pH 7.4) was injected into peritoneal cavity of an animal. Fifteen min later the peritoneal cavity was opened. Segments of small intestine was excised and immersed in the more fixative. The specimen was cut into small strips parallel to longitudinal axis of their submucosal vessels. The strips were then cut into blocks so that each block contained submucosal vessels. The blocks were then fixed in the ice-cold fresh fixative for 5 hours, washed several times in 0.1 M cacodylate buffer (pH 7.4) and stored overnight at 4°C. The specimens were subsequently fixed with 2% osmium tetroxide buffered with 0.1 M cacodylate solution (pH 7.4), dehydrated with graded ethanol and embedded in epon-epoxy resin. By light microscopy of thick sections stained with toluidine blue, 6 arteries in an outer diameter of 50–100 μ were randomly selected in each group. Thin sections of the each artery were then cut with diamond knives, stained with lead tartrate and examined with a JEOL JEM 100B electron microscope. The electron micrographs used for morphometric analysis of the arterial endothelium were originally taken at magnification of 30,000. Measurements of length were made on the micrographs with a vernier caliper, and those of area with Photo-pattern Analyser Model PPA (Applied Electric Lab Co, Tokyo, Japan) connected to X-Y Plotter Model F-3 (Riken Denshi Co, Tokyo, Japan). The following morphometric data were measured or counted; thickness of the endothelium, the number of pinocytotic vesicle, outer diameter of pinocytotic vesicle, frequency of pinocytotic vesicle and percentage of pinocytotic vesicle labeled with ferritin.

**Results**

Ultrastructural characteristics and changes in the endothelium of arterioles in the intestinal submucosa:

The arterioles were 80–100 μ in diameter at the mesenteric border of the intestine, diminishing to 50–60 μ in the submucosal region. The endothelium consisted of a single continuous layer of flattened cell and lay on a basement membrane which separated it from the internal elastic lamina. The cells
made contact and joined with one another along their narrow rims, forming intercellular junctions (Fig. 1).

There were no apparent changes in the ultrastructure of the endothelial cells and the intercellular junctions of the endothelium in each of the experimental group as well as in the control (Fig. 2).

Ferritin was present in high concentration in the blood plasma, located in pinocytotic vesicles and multivesicular bodies in the endothelial cells and also occurred in the subendothelial spaces in each experimental group. Ferritin concentration in the subendothelial space in the lysosomal fraction group was the highest among the experimental groups (Fig. 3). However, ferritin was never found in the interendothelial clefts in all the animals receiving pressor agents as well as saline (Fig. 2).

Morphometric data concerning vesicular transport of the arterial endothelium:

It is known that frequency of pinocytotic vesicles in capillary endothelium was inversely proportional to the thickness of endothelium. This was also
confirmed in the arterial endothelium in the control group (Fig. 4). Morphometric data were summarized in Table I. The 4 experimental groups resembled each other in the thickness of the endothelium and the outer diameter of pinocytotic vesicle. Frequency of pinocytotic vesicles per μ² of the endothelial cytoplasm was rather high in lysosomal fraction group. However, the value was statistically insignificant comparing to that of the other experimental groups. In lysosomal fraction group pinocytotic vesicles were most frequently labeled with ferritin. Percent of the labeling was 3.85, which was significantly high value in comparison with that in any of the other groups (p<0.01).

**DISCUSSION**

Winternitz et al\(^{10}\) induced serous effusion, necrotizing arterial lesions and hypertension in dogs by bilateral ligation of renal arteries and also by injection of renal extract to bilaterally nephrectomized animals. By protein analysis of the serous effusion, Nairn et al\(^{11}\) suggested that the effusion was the results of plasma leakage from the vascular system. Asscher and Anson\(^{12}\)
observed that animals receiving renal extracts showed concurrently a fall in plasma volume and a rise in venous hematocrit suggesting leakage from the vascular compartment. These results derived the concept of a release from damaged kidney tissue of humoral substances to injure the arterial walls and/or to increase the vascular permeability. The results also present 2 problems. The first is identification of the humoral substance(s) especially in relation to renin and angiotensin. The second is the mechanism how the humoral substance(s) causes the arterial necrosis; whether by its direct action, or by via high blood pressure, or by increased vascular permeability.

Cuthbert and Peart\(^{13}\) tried to purify the substance(s) of renal origin by using its nature of raising venous hematocrit as an assay system. They reported

Table I. Morphometric Results Concerning Vesicular Transport in the Arteriolar Endothelium

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Saline (n=25)</th>
<th>P.E. (n=23)</th>
<th>Ang. II (n=20)</th>
<th>K.E. (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of endothelium (µ)</td>
<td>0.26±0.02</td>
<td>0.28±0.02</td>
<td>0.31±0.02</td>
<td>0.29±0.02</td>
</tr>
<tr>
<td>No. of pv counted</td>
<td>472</td>
<td>561</td>
<td>722</td>
<td>1,038</td>
</tr>
<tr>
<td>Outer diamet of pv (Å)</td>
<td>755±95</td>
<td>762±90</td>
<td>730±86</td>
<td>758±92</td>
</tr>
<tr>
<td>Frequency of pv (µ²)</td>
<td>47.4±3.1</td>
<td>44.5±2.6</td>
<td>46.0±2.2</td>
<td>51.5±5.5</td>
</tr>
<tr>
<td>Pv labeled with ferritin (%)</td>
<td>1.48</td>
<td>1.60</td>
<td>1.52</td>
<td>3.85*</td>
</tr>
</tbody>
</table>

The values represent mean±S.E.
* p<0.01 (vs Saline, P.E., Ang. II, and K.E.)

Abbreviation; n= no. of electron micrographs used for analysis, P.E.= phenylephrine, Ang. II= angiotensin II, K.E.= Kidney extract, lysosomal fraction, pv= pinocytotic vesicle.
that the vascular permeability factor resembled the renin in respect of pressor activity, heat-lability, pH range of activity and molecular size, and also demonstrated that vascular permeability and pressor activity of the purified substances ran parallel and could not be dissociated. It was also suggested that the increase in blood pressure may be causally related to the increase in permeability. They, however, did not describe whether arterial necrosis occurred or not in their experimental animals. On the other hand, when equipressor doses of renal extract with high renin content and angiotensin were administered into bilaterally nephrectomized rats, Onoyama et al reported that arterial necrosis as well as serous effusion easily occurred in rats with the extract. The results suggest that the renal extract may exert the direct action on vascular system inducing the arterial necrosis and/or increased vascular permeability. The same renal extract as in the study of Onoyama et al was used in the present experiment.

To establish how the renal extract, renin or angiotensin are participated with the development of arterial necrosis, several investigations have been carried out by using a model of administration of the agents into bilaterally nephrectomized animals. Giese showed that angiotensin produces an acute elevation of blood pressure and results in the development of alternating segments of constriction and dilatation in mesenteric arteries. The dilated segments of the arterial trees exhibit necrosis and increased permeability, as manifested by the intramural deposition of intravenously injected carbon particles. Goldby and Beilin, on the other hand, observed that the degree of carbon deposition is not related to the nature of pressor substances such as noradrenalin, angiotensin, and renal extract, but to the height of blood pressure. There is a correlation between the mean blood pressure during a 4 hours experimental period and the percentage of arterioles labeled with carbon particles. Frequency of the intramural carbon deposit sharply increases when the mean blood pressure is above 150 mmHg. Since carbon particles do not transverse vascular endothelium in physiological condition, their deposition within the subendothelium indicates an occurrence of pathologic pathway or a gap. They concluded that acute severe hypertension per cent was responsible for pathologic pathway, which resulted from endothelial necrosis. Degree and duration of hypertension used in the vascular labeling experiments, therefore, may be too severe to evaluate a possible effect of pressor agents on physiological pathways of the arterial endothelium.

It is known that intercellular junctions of the arterial endothelium in the intestinal submucosa have a gap, and that peroxidase, a protein with a diameter of 40 Å, passes easily through the gap junction even in the physiological condition. Ferritin, a protein 110 Å in a diameter and comparable
in size to most plasma protein, does never penetrate into and pass through the gap junction of the arterial\textsuperscript{5} and capillary endothelium.\textsuperscript{1,9} The fact was confirmed not only in the arteries in the intestinal submucosa of the control animals, but also in those of the experimental animals receiving the pressor agents. Necrosis and separation of the intercellular junction did not occur in arterial endothelium of all the experimental animals. This means that pathological route of ferritin across the endothelium did not present in the present experiment, and that the degree and the duration of hypertension were suitable for evaluating the effect of the agents on physiological pathway of ferritin across the endothelium.

Macromolecules such as plasma protein pass across both the arterial and capillary endothelium by vesicular transport and reach the subendothelial space.\textsuperscript{4,1,9} In the present experimental animals ferritin particles in the arterial lumen were engulfed by caveolae intracellulaires, transported by pinocytotic vesicles and discharged into the subendothelial spaces. Ferritin concentration in the subendothelial spaces was the highest in lysosomal fraction group among the 4 experimental groups. Since the passage of ferritin through intercellular junctions of the endothelium was not observed, the main route of ferritin leakage was considered to be vesicular transport. Therefore, vesicular transport in the endothelial cells were analysed by morphometric technique. Morphometric analyses on the vesicular transport and movement of ferritin in the capillary endothelium were made in details by Bruns and Palade.\textsuperscript{9,19} Those in arterial endothelium, however, have been few. The outer diameter of the pinocytotic vesicles in each group ranged from 730 to 775 Å, and was very similar to that in capillary of 750 Å.\textsuperscript{9} The frequency of pinocytotic vesicles per $\mu^2$ of the arterial endothelium was very similar in each experimental group. When calculated by assuming an average thickness of 500 Å for each section, the frequency of the vesicles per $\mu^3$ ranged from 900 to 1,040, and was approximately identical to that of capillary endothelium, 442–1,310/$\mu^3$.\textsuperscript{9} The occurrence of labeled vesicles with ferritin was estimated to be about 10% in capillary endothelium.\textsuperscript{19} The ferritin labeling in arterioles of the present study was very low comparing to that in capillary. Some of the reasons for the difference are considered; these are differences in doses of ferritin injected, ferritin concentration of the solution used and organs observed. However, the most important one appeared to be difference in permeability per se between arteries and capillaries. The percentage of labeled vesicles with ferritin in lysosomal fraction group was the highest among the experimental groups. These results showed that the increase in labeling rate of pinocytotic vesicles with macromolecules was responsible for an increase in plasma insudation found in lysosomal fraction group. There was
neither difference in ferritin leakage into subendothelial spaces, nor in activity of vesicular transport in endothelial cells between phenylephrine or angiotensin group and control. The results indicate that increase in plasma insudation observed in lysosomal fraction group may not entirely be mediated by angiotensin or phenylephrine. It is suggested that substance(s) contained in lysosomal fraction originating from renal cortex might exert direct action on arterial endothelium and may result in increase of plasma insudation by accelerated vesicular transport.

References