ACUTE ARTERIOLAR LESIONS OF RAT INTESTINE CAUSED BY KIDNEY EXTRACT

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When administrated into bilaterally nephrectomized rats, kidney extracts produced angionecrosis as well as acute hypertension and serous effusion, which resembled those found in human malignant hypertension.\(^1\)\(^-\)\(^7\) The lysosomal fraction obtained from renal cortex of rats according to the method of Shibko and Tappel\(^8\) had a high renin activity,\(^9\) which produced fibrinoid necrosis of small arteries and arterioles in anephric rats.\(^10\) The same fraction also caused an increase in permeability in capillaries\(^11\) and arterioles.\(^12\)

Angiotensin II was also reported to produce medial necrosis of mesenteric arteries of rats with intact kidney\(^13\)\(^,\)\(^14\) or bilaterally nephrectomized rats.\(^4\)\(^,\)\(^15\) The extent of the medial changes was variable and depended upon the dose of angiotensin used and thereby the duration and severity of hypertension produced.

The present study is concerned with an electron microscopic observation of early vascular changes in the media of anephric rats injected with the lysosomal fraction of high renin activity. The finding was compared with that observed in the same type of animals injected with equi-pressor dose of angiotensin II.

MATERIALS AND METHODS
Female Wistar-King rats weighing about 200 gm were used. All the animals were anesthetized by intraperitoneal injection of amobarbital sodium (100 mg/kg), and the experiments were performed under the anesthesia. In order to exclude any participation of endogeneous renin-angiotensin system\(^4\) or a sustained pressor response to angiotensin and kidney extract\(^15\) bilateral nephrectomy was performed one hour before administration of the pressor agents. Mean arterial pressure was recorded from carotid artery through a cannula using a strain-gauge pressure transducer. As a pressor agent, synthetic angiotensin II amide (Hypertensin Ciba) in a dose of 0.08 \(\mu\)g/kg/min or 5.0 mg/ml of lysosomal fraction showing high renin activity,\(^9\) which was obtained from renal cortex of the normal rat by stepwise centrifugation method,\(^8\) was injected into the experimental animals. The above dose of each pressor agent caused sustained elevation of 20 mmHg in mean arterial pressure for 60 min as described in previous reports\(^11\)\(^,\)\(^12\) Five min prior to the injection of pressor agents, 0.55 ml/100 g body weight of 10% ferritin solution was injected into the femoral vein as a tracer.

Five animals were used in each experimental group. Five rats infused with 0.15 ml/100 gm body weight of physiological saline and also injected with 0.55 ml/100 gm body weight of 10% ferritin solution were served as controls. Sixty min after starting the administration of pressor agents, 20 ml of Karnovsky's fixative\(^16\) was injected into the peritoneal cavity of each animal. Fifteen min later the peritoneal cavity was opened, and small intestine was excised and immersed in the more fixative. The specimens

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were subsequently fixed in 2% OsO₄ solution containing 0.1 M sodium cacodylate (pH 7.4). All the tissue blocks were then dehydrated with graded ethanol and embedded in epon epoxy resin. By light microscopy of thick sections stained with toluidine blue, arterioles in the intestinal submucosa were selected in each group. Thin section was then cut with a diamond knife, stained with lead tartrate and examined with a JEOL JEM 100B and a Hitachi HU12 electron microscope.

RESULTS
Light Microscopy
Light microscopic observations of thick sections revealed no evidence of arteriolar lesions in the lysosomal fraction of kidney extract as well as in control and angiotensin II group.
Electron Microscopy
1) Control group: The ultrastructure of the arterioles was similar to that of previous descriptions. The arterioles consisted of a single layer of endothelial cells, an internal elastic lamina and one or two layers of smooth muscle cells. There were no ultrastructural changes in the arterioles (Fig. 1). The adventitia lacked an external elastic membrane, and consisted of loose connective tissue with collagenous and elastic fibers and a few fibroblasts (Fig. 1).

Ferritin particles were rarely seen in the subendothelial space but not in the interstitial space in the media.
2) Angiotensin II group: Endothelial cells and internal elastic lamina appeared to be intact. Most of the smooth muscle cells in the media showed findings of contraction, which were manifested by a decrease in the size of the cells, an increase in nuclear indentations, prominence of attachment devices and irregularity of the contours of the cells (Fig. 2).

The minimal changes characterized by lysis of the cell were occasionally seen in the smooth muscle cells. The lytic process was limited to a portion of a single muscle cell (Fig. 2 and 3). Irregularly shaped smooth muscle fragments of variable size were demarcated with cell membranes from the cell body. They lost the usual component of cytoplasmic structure and filaments and showed degenerative appearance (Fig. 3). Interstitial space of the media in the neighborhood of the lytic changes was enlarged. In some areas of these spaces collagenous fibrous
Fig. 2. An arteriole in the intestinal submucosa of a rat receiving angiotensin II. Smooth muscle cells (Sm) manifest contraction. Cytolysis is seen in a part of cytoplasm of a smooth muscle cell (arrows). At: attachment device. × 13,000.

Fig. 3. A lytic process of a smooth muscle cell in angiotensin II group. Fragments (Fr) of cytoplasm of the muscle cell are separated by apposed two cell membrane (cm1, cm2) from cell body and lose their usual cytoplasmic component. Collagenous and elastic fibers are seen in the interstitial space. × 53,000.
Fig. 4. Media of an arteriole of a rat receiving lysosomal fraction of kidney extract exhibits a necrotic lesion. In the necrotic focus muscle fragment (Mr), cell debris (Cd), small vesicle (Sv) and fibrous element (Fi) are seen within basement membrane-like ground substances (Bm). Some area of the focus appears to be edematous (Ed). Sb. basement membrane-like substances in the subendothelial spaces. × 30,000. Insert in an upper corner: vesiculation of smooth muscle cell (Sm). × 85,000. Insert in a lower corner: fibrous component in the necrotic focus. Arrow shows a ferritin particle. × 80,000.
structure and elastin were occasionally seen (Fig. 3).

Ferritin particles appeared occasionally in the subendothelial spaces but rarely in the media (Fig. 3).

3) Lysosomal fraction group: Endothelial cells appeared apparently to be intact. In the subendothelial spaces, however, there was an increase in the amount of basement membrane-like substances. Internal elastic lamina showed focal disruption (Fig. 4).

The most severe lesion occurred in the foci of media. In these foci smooth muscle cells decreased in their size and were fragmented into small pieces of the cells, being displaced by amorphous, basement membrane-like ground substances. Cell fragments, cell debris, collagenous fibers and elastin were embedded in these ground substances. The most characteristic finding in the necrotic foci was occurrence of small vesicles in neighboring regions of the necrotizing muscle cells. These vesicles which were surrounded by a unit membrane were round in shape and were about 0.1 μ in diameter (Fig. 4 insert).

In some areas of the basement membrane-like ground substances there was a decrease in electron opacity appearing to be edematous. In these edematous regions a few ferritin particles were sparsely distributed (Fig. 5).

The adventitia became more loose and showed edematous appearance (Fig. 4).

DISCUSSION

We previously reported that arterioles of the intestinal submucosa exhibited focal areas of fibrinoid necrosis 20 to 24 hours after bilateral nephrectomy. In the present study, however, there were no ultrastructural abnormalities of the arterioles in the intestine of control rats 2 hours after the bilateral nephrectomy. On the other hand, the arterioles of the animals receiving angiotensin II or kidney extract showed a variable extent of ultrastructural changes in their media. It is, therefore, reasonable to consider that these medial changes were attributed to an elevation of arterial blood pressure induced by these pressor agents. The medial changes, however, could not be recognized by light microscopy. Since mean arterial pressure of the rats was raised only about 20 mmHg for short duration of 60 min, these changes were considered to represent quite earlier changes in the hypertensive vascular lesions.

In both groups of animals receiving either angiotensin II or the lysosomal fraction cytolytic

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changes were observed in the medial muscle cells. The cytolysis found in angiotensin II group was limited to single cells. Some areas of the cytoplasm were demarcated by cell membranes from the cell body and were fragmented into a variety of cell mass. These findings were essentially identical to the observations in mesenteric arteries of rats with angiotensin-induced hypertension by Wiener and Giacomelli. As a mechanism in such cytolysis they considered an invagination of cell membranes into cell bodies resulting in a demarcation of focal cytoplasm from the cell bodies. The same mechanism can be applied to the focal cytolysis in angiotensin II group of the present study.

The extent of cytolysis was more pronounced in lysosomal fraction group than that in angiotensin II group. Fragmentation and necrosis of smooth muscle cells were more advanced and some areas of the media lacked the muscle cell and were focally displaced by ground substances consisting of basement membrane-like materials, fibrous components and cell debris. In addition to the focal cytoplasmic fragmentation probably induced by the invagination of cell membranes, another mechanism of cell degradation appeared to occur in the lysosomal fraction group. A large number of round vesicles about 0.1 μ in diameter which were surrounded by a cell membrane were observed in neighboring region of the necrotizing muscle cells. This finding suggests that these small vesicles containing cytoplasmic constituents were discharged from the necrotizing cells by membrane vesiculation resulting in a decrease in the cell size. The occurrence of these small vesicles was reported in arterial lesions of the experimental animals with chronic hypertension but not in those in angiotensin-induced hypertension.

The present study revealed some differences in the extent and features of ultrastructural changes in the media between angiotensin II and lysosomal fraction group. The most characteristic change found in lysosomal fraction group, however, was an appearance of ferritin particles in edematous foci of the necrotizing media. The ferritin leakage into the media can be explained by an increased permeability found in the arteriolar endothelium in this group. Since the ferritin molecules are known to be about 110 Å in diameter and are comparable in size to most plasma proteins, the findings suggest occurrence of abnormal leakage of plasma constituents into the necrotizing media. Increased plasma insudation into the media was considered to be a cause of the necrosis of smooth muscle cell. Though the arteriolar vasculature received equal or similar pressor effect, the differences in the medial necrosis may be explained by those in vascular permeability between angiotensin II and lysosomal fraction groups.

The present study indicates that the vascular changes found in lysosomal fraction group (high renin content) were not merely mediated by angiotensin II. It is not known, however, what substance(s) caused both the medial necrosis and increased permeability in the arteriolar endothelium. Renin per se or the other factors originating from kidney tissues not mediated by angiotensin II may be involved in the production of the vascular lesions.

SUMMARY

Lysosomal fraction of renal cortical extract, which showed high renin activity, and equipressor dosis of synthetic angiotensin II amide were administrated into one-hour nephrectomized rats. Sixty minutes after the sustained elevation of 20 mmHg in mean arterial pressure by each pressor substance, the rats were sacrificed and the intraperitoneal organs were fixed. Five minutes prior to the administration of each pressor substance ferritin solution, as a test substance for vascular permeability, was intravenously injected. Medial changes in the arterioles in the intestinal submucosa were observed by electron microscopy. In angiotensin II group early lytic lesions of the muscle cells were limited to the single muscle cells. Ferritin particles were rarely found in the media. In lysosomal fraction group the lytic lesions were more advanced. Some regions of the media exhibited focal loss of smooth muscle cells manifesting focal medial necrosis. Ferritin particles were distributed in some areas of the necrotic media. The results suggested that the kidney extract with high renin content contained substance(s) to produce both medial necrosis and plasma insudation into the media of the arterioles.

REFERENCES

2. NAIRN, R. C., MASSON, G. M. C., & CORCORAN, A. C.: The production of serous effusions in nephrectomised animals by the administration of renal extracts and renin. J. Path. Bacteriol. 71:

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