Scanning Electron Microscopic Study of Endothelial Cells of Cerebral Arteries from Spontaneously Hypertensive Rats

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SUMMARY Endothelial cells of the cerebral arterial system in spontaneously hypertensive rats were investigated by scanning electronmicroscopy and found to show progressive changes such as increased microvilli, numerous plasmalemmal pits, enlargement of the cells and well-developed marginal folds. Regressive changes, such as balloon-like protrusions and crater-like cave-ins, were also observed. Platelet adhesion to the injured endothelial surface of cerebral arteries was frequent. The significance of these changes in the development of hypertensive cerebrovascular lesions is discussed.

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SPONTANEOUSLY HYPERTENSIVE RATS (SHR), as developed by Okamoto and Aoki,1 show various vascular lesions, which can involve cerebral vessels and cause cerebral bleeding, necrosis and edema.2,3 Cellular hyperplasia and hyaline degeneration of pial arteries and intracerebral arterioles, as well as fibrinoid necrosis, microaneurysm and thrombosis of intracerebral arterioles, represent the underlying vascular changes in the injured brain of SHR.4,5 These vascular changes imply that functional and organic arterial endothelial changes, inducing increased vascular permeability, play important roles in the development of lesions in both vessels and brain parenchyma.6,7 The present study was attempted to obtain further information concerning changes in endothelial cells of the cerebral arteries in SHR.

Materials and Methods

Nine male SHR of the A8 substrain (stroke-prone) (SHRSP) and 8 control male rats of Wistar Kyoto strain (WK) ranging in age from 8 to 52 weeks were used. Systolic blood pressure was measured by the tail pulse pick-up method. Under sodium pentobarbital anesthesia animals were perfused through the left ventricle with saline at 37°C, followed by 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The excised basal arteries of the brain were further immersed in the same fixative for one hr, washed in 0.1 M phosphate buffer (pH 7.4) 3 times and post-fixed in 1% OsO4 in the same buffer solution for one hr. After specimens were passed through isoamyl acetate, dried by the critical point method, coated with gold and examined under a Hitachi scanning electron microscope HS-7D.

Results

Blood pressures of SHR and control animals are shown in the table. Scanning electronmicroscopy in the control animals showed a large number of microvilli on the luminal surface of the endothelial cells of the cerebral arteries at branching sites. In areas other than branching sites only a small number of microvilli on the endothelial cells were seen. In SHR, the endothelial cells on the cerebral arteries had a large number of microvilli distributed diffusely not only at branching sites but elsewhere (fig. 1).
### Table 1: Age and Blood Pressure of SHR and Control Animals

<table>
<thead>
<tr>
<th>Group</th>
<th>SHR</th>
<th>Control rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (weeks)</td>
<td>Number</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>20-24</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>35-40</td>
<td>5</td>
</tr>
</tbody>
</table>

In aged animals, these microvilli showed a tendency to become globular or knob-like, as a whole or at the apical portion of the projections (fig. 2).

In the control animals, the endothelial cells were generally flat, with slight elevations in the central portion of the cells. The endothelial cells at branching sites, however, protruded and appeared hemispherical. In SHR, protrusion of the endothelial cells at branching sites was more prominent than in the controls (fig. 3) and more enlarged cells were observed not only at branching sites but also in other areas.

A large number of pinholes was observed on the luminal surface of the endothelial cells in the control animals examined in highly magnified scanning electronmicrographs. The endothelial cells in SHR possessed more plasmalemmal pits than the control animals (fig. 4). Degenerated endothelial cells in SHR showed a definite increase in plasmalemmal pits on their luminal surface when compared with neighboring endothelial cells (fig. 4).

In SHR, well-developed, irregular marginal folds were observed on the surface of the endothelial cells (fig. 5). Such a structure was not seen in the control rats. Only a slight elevation was observed at the endothelial cell boundaries. Sometimes small depressions were encountered between endothelial cell junctions in SHR, particularly in the aged animals.

In SHR, particularly in the aged animals, balloon-like protrusions of the endothelial cells were often observed (fig. 6). Deformed microvilli, globular or knob-like, were seen in these protrusions but sometimes they were smooth. Crater-like cave-ins were also often observed in SHR (fig. 7). On the concave surface of the craters plasmalemmal pits and microvilli were noted. There were transitional forms between balloons and craters, and sometimes both changes were observed in the same endothelial cell (fig. 8). Similar craters and balloons were occasionally seen in the control animals but only at branching sites. Some endothelial cells in SHR showed multiple holes

![Fig. 1](http://stroke.ahajournals.org/)

*Fig. 1. A large number of microvilli observed on the endothelial surface of the SHR cerebral artery. Scanning electronmicrograph. SEM × 24,000.*
FIGURE 2. Microvilli with globular or knob-like changes of the endothelial cells of the cerebral artery in an aged SHR. SEM × 60,000.

FIGURE 3. Marked protrusion of the endothelial cell at a branching site of the cerebral artery in SHR. SEM × 9,000.
FIGURE 4. A large number of plasmalemmal pits on the endothelial surface of the SHR cerebral artery. The endothelial cell with partial crater-like change shows more plasmalemmal pits than the surrounding cells. SEM × 24,000.

FIGURE 5. Well-developed irregular marginal folds of the endothelial cells of the SHR cerebral artery. At the right end of the picture, a small crater is visible. SEM × 24,000.
FIGURE 6. A balloon-like protrusion of an endothelial cell of the SHR cerebral artery. Globular or knob-like changed microvilli are seen on the surface of balloon. SEM × 24,000.

FIGURE 7. A crater-like cave-in on an endothelial cell of the SHR cerebral artery. On the concave surface of the crater, microvilli are visible. SEM × 24,000.
FIGURE 8. Two balloons and a crater exist on the same endothelial cell of the SHR cerebral artery. SEM × 9,000.

FIGURE 9. On an endothelial surface there are multiple small holes through which a cavium-like vacuole of the cell is visible. SEM × 9,000.
on their surface (fig. 9). A necrotic mass of cells was occasionally seen attached to the surface of degenerating endothelial cells.

Adhesion of a small cluster of platelets was often observed on the endothelial cells of the cerebral arteries in SHR (fig. 10). Some of the adhered platelets showed pseudopods, while others were ovoid. Among these platelets there was frequently a small mass of amorphous substance. In some areas, endothelial injury was covered by the adherent platelets. Occasionally such an accumulation of platelets was seen around a crater or intercellular small recess.

**Discussion**

A large number of microvilli occurring diffusely, not just at branching sites, was one of the most obvious changes in the endothelial cells of the cerebral arteries in SHR. Transmission EM study has also revealed an increase of endothelial projections in the cerebral arteries in SHR, but the appearance was not so striking as in the present scanning EM study. Microvilli have been reported in various arteries described as "endothelial microvilli," "endothelial projections," and "hair-like projections." The function of these microvilli is unknown. Tokunaga et al. suggested that they may indicate an elevated function of the endothelial cells. Smith et al. regarded an eddying flow among the endothelial surface due to action of microvilli as significant in providing conditions of flow and pressure favorable for the exchange of metabolites. It is assumed that the uptake of plasma components into endothelial cells is augmented by the increased surface area of the cells. The presence of many microvilli on the endothelial cells of the cerebral arteries in SHR also may be an expression of the immaturity of the cells, because such profuse microvilli have been reported by Takeshige et al. in the fetal aorta, ductus arteriosus and pulmonary arteries.

The protruded endothelial cells with a hemispherical appearance observed in the scanning EM of the cerebral arteries in SHR seem to correspond to the enlarged activated endothelial cells with a large nucleus and rich in organelles observed in transmission EM. These enlarged cells are believed to be regenerated young endothelial cells replacing injured cells, because Wright has shown with autoradiographic study using H-thymidine that increased endothelial cell mitoses can be observed at branching sites where the enlarged endothelial cells are localized in normotensive rats. Our autoradiographic study with

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**FIGURE 10.** A small cluster of platelets adhering to the endothelial surface of the cerebral artery in an aged SHR. Fibrin mass (arrow) is seen among the platelets. SEM X 21,000.
"H-thymidine revealed an increase in labeled endothelial cells in the cerebral arteries of SHR as compared with control animals. The presence of a large number of pinholes or plasmalemmal pits on the luminal surface of the endothelial cells of the cerebral arteries of SHR suggests an increased vesicular transport in the cells. Our previous transmission EM study and horse-radish peroxidase experiments also revealed an augmented pinocytosis in the endothelial cells. It is an interesting finding that the degenerated endothelial cells possess definitely more plasmalemmal pits than neighboring endothelial cells. In the horse-radish peroxidase experiment, increased pinocytotic vesicles filled with peroxidase and a massive deposition of peroxidase were observed in the same medial smooth muscle cell in the media of the cerebral arteries in SHR. These facts strongly suggest that an increased vesicular transport, due to hypertension, causes intracellular edema or degenerative changes in the cells.

Well-developed, irregular, marginal folds were noted on the endothelial surface of the SHR cerebral artery. The function of these folds is also unknown. Transmission EM study revealed irregularity of the endothelial cell junctions, occasionally with partial detachment. This irregularity of the junctions seems to be associated with the rearrangement of the regenerated endothelial cells. Schwartz et al. showed that mechanical denudation of the aorta lining was followed by abnormal intercellular junctions even after re-establishment of a continuous endothelium.

In addition to the above-mentioned changes, regressive changes, such as balloon-like protrusions and crater-like cave-ins, were observed. The craters seem to be ruptured or collapsed balloons, as Kawamura et al. pointed out, because transitional forms of both conditions and the concave surface of craters occasionally revealed plasmalemmal pits and microvilli exactly as seen on the endothelial surface. Nelson et al. reported balloons and craters in ischemia and regarded these changes as a nonspecific reaction of endothelial cells to injury. The balloons and craters observed in the present study seem to be the direct sequela of hypertension rather than of secondary ischemia due to hypertensive vascular changes as they were found in the carotid artery and aorta where there was no evidence of narrowing of the lumen.

The present scanning EM study, as well as the previous transmission EM study revealed aggregation and adhesion of platelets to the endothelial cells of the SHR cerebral arteries. Collagen, subendothelial basement membrane, and microfibrils are known to have an affinity for platelets. Aggregation and adhesion of platelets to the injured endothelial surface leads to thrombus formation. The proliferating factor of platelets may also play a role in the development of cellular hyperplasia of the vessel wall.

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References

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