Permeability of Intracranial Extracerebral Vessels in Stroke-Prone SHR

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SUMMARY Permeability of intracranial extracerebral arteries of stroke-prone spontaneously hypertensive rats (SHRSP) was studied using labeling techniques (ferritin and horseradish peroxidase), at the cellular level.

In the arterial endothelial cells, the tracer molecules were slowly but constantly transported by the plasma-lamellar vesicles to the subendothelial space. This endothelial transportation of the tracers into these cerebral arteries did not seem to be significantly influenced by aging, increased blood pressure, hyperlipidemia or the existence of cerebral bleeding and infarction.

Around the adventitia, there were a great number of periadventitial capillaries, especially near bifurcations. In the periadventitial capillaries, the tracer molecules were readily trapped by endothelial cells and were quickly transported to pericapillary spaces. The tracer molecules were then detected in the phagocytes adjacent to the deeper layers of the media, and further in the medial smooth muscle cells. The possibility that large amounts of plasma components are supplied to the media from periadventitial capillaries in the intracranial extracerebral arteries has to be considered in the pathogenic mechanisms of cerebrovascular lesions.

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SPONTANEOUSLY hypertensive rats (SHR) serve as an excellent model for research on essential hypertension in humans. The pathogenetic mechanisms related to stroke have also been elucidated since the establishment of stroke-prone SHR (SHRSP). Cerebrovascular lesions develop spontaneously in over 80% of this species. Arteries in the cerebral lesions reveal hyaline degeneration, fibrinoid necrosis and thrombosis during the process of aging.

In acute and chronic experimental hypertension there is an increased cerebrovascular permeability to protein and dyes. In the process involved in the protein leakage it is difficult to assess as cells in the brain are joined by tight junctions which prevent protein tracers from entering the brain. In the present experiments, ferritin and horseradish peroxidase were used to study cerebrovascular permeability changes with the development of cerebrovascular lesions in SHRSP. Reported are the ultrastructural findings indicating a clear difference in the permeability of arterial and capillary endothelium.

Materials and Methods

Seventeen SHRSP from 28 to 40 weeks of age with symptoms of stroke (group 1) and 30 asymptomatic SHRSP from 8 to 40 weeks old (control group) were used. In addition, 15 SHRSP fed a high-fat-cholesterol diet (containing 20% suet, 5% cholesterol and 2% cholic acid) for 2 to 16 weeks (HFC group) were also used. Horseradish peroxidase (Type 11, Sigma Chemical Co., St. Louis, MO) and ferritin (6X crystallized, horse spleen, cadmium removed, Miles Laboratories, Elkhart, IN) were used as tracer molecules.

All rats in each group were anesthetized with pentobarbital. Either 10 mg/100 g body weight of peroxidase dissolved in physiologic saline or 80 mg/100 g body weight of ferritin dissolved in physiologic saline was injected into the femoral vein. Several rats in each group were injected with 0.2 ml/100 g body weight of physiologic saline only. Cerebral arteries were perfused with a fixative through the left ventricle of the heart, 3, 5, 10, 15 and 30 min after the peroxidase injection, and 5, 15, 30 and 60 min after the ferritin injection. The perfusion fixation was carried out at 180 mm Hg pressure for 4 to 5 min with a 2% formaldehyde-2.5% glutaraldehyde mixture in 0.1 M cacodylate buffer (pH 7.4) (Karnovsky, 1965) at room temperature. After the perfusion fixation, the brains of SHRSP were carefully removed and fixed with the same preparation, for 3 h at 4°C. The intracranial extracerebral arteries were then dissected as far as possible. The dissected arteries were fixed again with a 2% formaldehyde-2.5% glutaraldehyde mixture buffered with 0.1 M cacodylate buffer (pH 7.4) (Karnovsky, 1965) at room temperature. After the perfusion fixation, the brains of SHRSP were carefully removed and fixed with the same preparation, for 3 h at 4°C. The intracranial extracerebral arteries were then dissected as far as possible. The dissected arteries were fixed again with a 2% formaldehyde-2.5% glutaraldehyde mixture buffered with 0.1 M cacodylate buffer for 1 h at 4°C, and then washed overnight at 4°C in 0.1M cacodylate buffer. Afterward, the dissected arteries were cut into small pieces.

The sections, obtained from the rats injected with either peroxidase or physiologic saline, were washed briefly in cold, distilled water, and were then incubated in a buffered solution containing diaminobenzidine and hydrogen peroxide (Karnovsky, 1967). After incubation the sections were washed in 4 changes of distilled water over a period of about 10 min.

After post-fixation with 2% OsO₄ buffered with 0.1 M cacodylate buffer for 2 h, tissue sections were stained with 2% uranyl acetate in 50% ethanol for 1 h.
at 4°C, dehydrated in graded ethanol and embedded in Epon 812.

These sections were cut on a Sorvall Porter-Blum, MT-2B ultratome. Thin sections were now stained with lead citrate and uranyl acetate and were examined under a Hitachi HS-9 electron microscope.

Results

Ferritin Experiments

In the intracranial extracerebral arteries at 5 min after injection, ferritin molecules were not evident in the endothelial cells. Fifteen min after injection, a small number of ferritin molecules were detected within the plasmalemmal vesicles of the endothelial cells and in the subendothelial spaces. Small groups of ferritin molecules were seen within the dense bodies or multivesicular structures of the endothelial cells. These molecules were never detected within the interendothelial junctions. At 30 ~ 60 min after injection, the number of ferritin molecules within the plasmalemmal vesicles, dense bodies of the endothelial cells and subendothelial spaces increased only slightly (fig. 1). The ferritin molecules did not pass through the internal elastic lamina, and, consequently, were never seen in the media. These findings were generally common to the stroke group, the control group and the HFC group. Intravenously given ferritin molecules were slowly but constantly transported to the subendothelial space by the plasmalemmal vesicles. The endothelial transportation of the tracers into these intracranial extracerebral arteries did not seem to be significantly influenced by aging, increased blood pressure, hyperlipidemia or the existence of cerebral bleeding and infarction.

In the outer layers of the media, numerous perivascular capillaries and phagocytes were seen just adjacent to the adventitia. In the stroke group at 5 min after injection, ferritin molecules were seen within the plasmalemmal vesicles and dense bodies of the perivascular capillary endothelium. At 15 min after injection, ferritin molecules had penetrated the endothelial cells and were seen in the phagocytes, especially within the dense bodies, next to the adventitia. At 30 and 60 min after injection, numerous ferritin molecules were trapped by the phagocytes and accumulated in their dense bodies. In the control and HFC groups, the number of ferritin molecules, trapped by the phagocytes, increased only slightly at 30 and 60 min after injection. Therefore, capillary endothelial transportation of the tracers was evidently increased in the stroke group.

Peroxidase Experiments

The intracranial extracerebral vessels, incubated in the medium containing diaminobenzidine after the in-
jection of physiologic saline only, had no reaction products either in the endothelium or in the adventitia.

In the intracranial extracerebral arteries at 3 min after the injection of horseradish peroxidase, peroxidase molecules were seen on the luminal surface of the endothelial cells and within some of the plasmalemmal vesicles. At 10 and 15 min after injection, a small number of peroxidase molecules appeared in plasmalemnal vesicles at the subendothelial surface of the endothelial cells and also in the subendothelial space. At 15 and 30 min after injection, the number of peroxidase molecules increased slightly within the plasmalemnal vesicles and dense bodies of the endothelial cells as well as in the subendothelial spaces. The peroxidase molecules, which lay scattered in small quantities in the subendothelial space, did not penetrate the internal elastic lamina. Peroxidase molecules were never seen within interendothelial junctions. These findings were generally common to the stroke, control and HFC groups.

We then examined the deeper layers of the media and the adventitia. At first, a great number of periadventitial capillaries and phagocytes were found just adjacent to the adventitia, particularly around bifurcations. In the stroke group at 3 min after injection, numerous peroxidase molecules were evident within the plasmalemnal vesicles and dense bodies of the periadventitial capillary endothelium. Some of the peroxidase molecules were rapidly transported to the pericapillary space by the plasmalemmal vesicles. Afterward, the molecules were trapped by the pericytes and the phagocytes adjacent to the deeper layers of the media. Serial thin sections revealed that the plasmalemmal vesicles were frequently connected and they appeared as chains which formed channel-like structures across the endothelium. At 5 and 10 min after injection, massive numbers of peroxidase molecules were trapped by the phagocytes and accumulated in the dense bodies (fig. 2). At 10 min after injection, small groups of peroxidase molecules were frequently seen within the plasmalemmal vesicles and dense bodies of the medial muscle cells. These muscle cells were found near the phagocytes with massive peroxidase-containing dense bodies (fig. 3). At 15 and 30 min after injection, massive numbers of peroxidase molecules were found in the smooth muscle cells, especially within dense bodies, just beneath the internal elastic lamina (fig. 4). In the control and HFC groups, a small number of phagocytes with peroxidase-containing dense bodies were also found next to the media. In these groups, the number of peroxidase molecules seemed to increase slightly, and with the passage of time a small number of peroxidase molecules were trapped by the media smooth muscle cells.

The peroxidase molecules, found in the smooth muscle cells, did not seem to be transported from the arterial lumen as there was no morphological evidence.

**Figure 2.** Middle cerebral artery of SHRSP (stroke group) at the age of 34 weeks. a. A large amount of peroxidase reaction products is evident in a phagocyte (Ph) just next to a periadventitial capillary (Ca). Thick and thin arrows indicate the dense bodies of the phagocyte. Five min after injection of peroxidase. EC: endothelial cell. SM: smooth muscle cell. × 7,200. b. Higher magnification of the area indicated by the thick arrow. Massive peroxidase reaction products are seen within a dense body (DB). × 70,000. c. Higher magnification of the area indicated by the thin arrow. DB: dense body. × 70,000.
that the peroxidase molecules, scattered in the subendothelial space, had penetrated the internal elastic lamina. These ultrastructural findings suggest that a great number of the plasma components were supplied to the media from the periadventitial capillaries.

Discussion

Physiologic studies of the permeability of capillaries led to the "pore theory" which assumes the existence of 2 systems, one with small and the other with large pores. The morphologic equivalent for the small-pore system may be the endothelial cell junctions, and for the large-pore system, the plasmalemmal vesicles. Investigators have directed their attention to the endothelial cells. Insinuation of plasma proteins into the arterial wall and increased permeability to different tracers have been reported in experimental hypertension. A tracer study in experimental hypertension showed peroxidase passage through cerebral artery endothelial junctions, with no leakage of carbon particles. Another experiment showed increased permeability of cerebral vessels in either of 3 different pathways, i.e., through channels in the cytoplasm, transendothelial pinocytosis and endothelial cell junction.

The present study revealed a small number of both ferritin and peroxidase molecules within endothelial plasmalemmal vesicles in the intracranial extracerebral arteries. The number of these molecules within the plasmalemmal vesicles and dense bodies of the endothelial cells, as well as in the subendothelial space, increased only slightly with the passage of time. Neither ferritin nor peroxidase molecules were detected within the inter-endothelial junctions. These observations were similar in the stroke, control and HFC groups. The endothelial transportation of the tracers in these arteries did not seem to be significantly influenced by aging, increased blood pressure, hyperlipidemia or the existence of cerebral bleeding and infarction.

These findings raise doubts that there is an increased permeability of arterial endothelium to plasma protein in hypertension, and also doubts as to the role of the inter-endothelial pathway (small-pore system). The tracer molecules were probably slowly but constantly transported by endothelial plasmalemmal vesicles (large-pore system), and the substance transport through the endothelium did not play a leading part in these intracranial extracerebral arteries.

In a large-sized artery, substances enter the arterial wall from 2 directions: from the lumen and from the...
FIGURE 4. Middle cerebral artery of SHRSP (stroke group) at the age of 34 weeks. a. A large amount of peroxidase reaction products in the smooth muscle cells, especially within dense bodies (thin and thick arrows), just beneath the internal elastic lamina (IEL). Fifteen min after injection of peroxidase. EC: endothelial cell. SM: smooth muscle cell. AD: adventitia. × 7,200.

b. Higher magnification of the area indicated by the thick arrows. Massive peroxidase reaction products are seen within the dense body (DB). × 120,000.

periadventitial capillaries. The periadventitial capillaries of cerebral arteries have not been examined closely because cerebral arteries are small or medium-sized and periadventitial capillaries were not considered to play an important role. In our experiment, we directed our attention to the adventitia and the deeper layers of the media and found that a great number of periadventitial capillaries were adjacent to and surrounding the adventitia, especially around bifurcations, in the intracranial extracerebral arteries. We showed that in the periadventitial capillaries the tracer molecules were promptly trapped by capillary endothelial cells, and thereafter were transported to the pericapillary spaces. The phagocytes adjacent to the deeper layers of the media trapped tracer molecules leaked from the capillary lumen. Especially in the stroke group, a large number of tracer molecules accumulated in the phagocytes in contrast to the endothelial cells of intracranial extracerebral arteries in which only a few tracer molecules were seen. Near the phagocytes with massive tracer molecules, smooth muscle cells of the deeper layers of the media also trapped tracer molecules. With the passage of time, tracer molecules were found in all layers of the media. Our results strongly suggest that in the cerebral arteries, large amounts of plasma substances are supplied to the media from the periadventitial capillaries.

It has been suggested that the structural basis for increased capillary permeability is a transient opening of inter-endothelial junctions caused by chemical mediators producing either endothelial contraction or affecting the protein-polysaccharide complexes at the inter-endothelial junction. Eto et al. have found that kidney extract increases capillary permeability for plasma proteins, at least via increased vesicular transport. Simionescu et al. have showed transendothelial channels in the capillary and Brightman has suggested a passive transfer of protein through actively formed channels in the cerebral capillary endothelium since peroxidase was injected postmortem. Lossinsky et al. have presented cytochemical evidence for endothelial channel-lysosome connections. Our study showed the lack of tracer molecules within the inter-endothelial junctions, while massive amounts of tracer molecules were observed within plasmalemmal vesicles in the periadventitial capillaries. The plasmalemmal vesicles were frequently connected and they appeared as channels across the endothelium. These findings indicate that vesicular transports or transendothelial channels may play an important role in increased periadventitial capillary permeability. The possible disturbance of periadventitial capillary permeability in the initiation of intracranial extracerebral arterial lesions must be considered in hypertension.
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References

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