Ultrastructural Changes in Cerebral Pericytes and Astrocytes of Stroke-Prone Spontaneously Hypertensive Rats

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We examined the ultrastructure of cerebral pericytes and astrocytes in 20 normotensive Wistar-Kyoto rats and 60 asymptomatic stroke-prone spontaneously hypertensive rats killed at 4-52 weeks of age. Another 30 stroke-prone spontaneously hypertensive rats were killed soon after they showed symptoms of stroke. We found two kinds of pericytes around the capillaries: granular pericytes and filamentous pericytes. Granular pericytes possibly serve as scavenger cells in the central nervous system and became active and grew in size with time. In contrast, filamentous pericytes degenerated during the development of hypertension. Degeneration of the filamentous pericytes was involved in an increase of endothelial permeability. Increased permeability caused focal and then circumferential swelling of the astrocytes around the capillaries. Swelling of the astrocytes seemed to accelerate the production of attachment plaques. Following this increase in the number of attachment plaques, numerous astrocytic filaments were produced within the cytoplasm. As a result, fibrous astrocytes were fully developed. Adjacent to the fibrous astrocytes we detected opening of the interendothelial junctions as well as dead neurons. From these observations we propose that astrocytes perform the main function in trophic interactions among cerebral endothelial cells, astrocytes, and neurons during chronic severe hypertension in SHRSP.

**Materials and Methods**

Okamoto and colleagues developed SHRSP, in which stroke (cerebral hemorrhage and/or infarction) develops spontaneously in >80%; the cerebral lesions resemble those found in humans. Since the development of this rat strain we have continued to inbreed them selectively, and at present we have the main colony of this inbred strain. There are more than 1,500 of these rats in our laboratory. Thirty SHRSP aged 28-50 weeks with symptoms of stroke made up the stroke group, 60 asymptomatic SHRSP aged 4-52 weeks made up the asymptomatic group, and 20 normotensive Wistar-Kyoto rats (WKY) aged 4-50 weeks made up the control group. All rats in each group were anesthetized with pentobarbital, and the cerebral vessels were perfused with 1% formaldehyde and 1.25% glutaraldehyde in 0.1 M cacodylate buffer via the descending aorta; after perfusion the brains were carefully removed, sliced at the coronal section, and fixed with 2% formaldehyde and 2.5% glutaraldehyde in the same buffer as described previously. The brain tissues were washed overnight at 4°C in 0.1 M cacodylate buffer; they were then sectioned at 0.5 mm thicknesses and 2.5 mm widths under a microscope.

For cytochemical studies, several sections from each rat in the three groups were cut on a tissue chopper without freezing and were collected into 0.1
M cacodylate buffer. The sections were then incubated for visualization of acid phosphatase activity according to a modified Gomori method.

For tracer studies, 10 mg/100 g body wt horseradish peroxidase (Type II, Sigma Chemical Co., St. Louis, Mo.) was injected into a femoral vein of five symptomatic SHRSP aged 20–28 weeks, eight asymptomatic SHRSP aged 8–24 weeks, and six WKY aged 12–24 weeks. After the peroxidase injection, the cerebral arteries were perfused and the brains were removed, fixed, and sliced as indicated earlier.

For morphologic studies, all sections were post-fixed with 2% OsO₄ buffered with 0.1 M cacodylate buffer for 2 hours. The sections were then stained with 2% uranyl acetate in 50% ethanol for 1 hour at 4° C, dehydrated in a graded series of ethanol, and embedded in Epon 812. Semithin sections were stained with toluidine blue for light microscopy, and ultrathin sections were either unstained except for the original section staining with uranyl acetate or double-stained with uranyl acetate and lead citrate for electron microscopy, as described previously.

Furthermore, for the morphometric study we took electron micrographs of at least 100 capillaries from five asymptomatic SHRSP and five WKY aged 12 weeks and from five asymptomatic SHRSP and five WKY aged 24 weeks. The capillaries examined were cut transversely and had an inner radius of 5–8 μm. We measured the length of the endothelial basement membrane (L₁) as well as the length of the basement membrane that was lined with pericytes (L₂). We divided L₂ by L₁ to give the pericyte lining ratio. Based on their lining ratios, we divided the capillaries from each rat strain into four groups (those with lining ratios of 0–25%, 25–50%, 50–75%, and 75–100%). The χ² test was used to compare the percentages of the total capillaries from SHRSP and WKY in each lining ratio group.

Results

From each WKY, we obtained at least 10 cerebral sections from the anteromedial and occipital cortices. Morphologic studies revealed that the cerebral capillaries were surrounded by two kinds of pericytes. One kind had filaments (filamentous pericytes) and the other had inclusion bodies (granular pericytes). Filamentous pericytes contained dense bands of filaments, some of which were located beneath the plasma membranes and in peripheral extensions of the cytoplasm. All cerebral capillaries were covered with the filamentous pericytes, which were thin but morphologically well maintained. Granular pericytes were found around bifurcations of the capillaries and were very small (approximately 5 μm in diameter) in WKY aged 4 weeks. Detected in one of every 100 capillaries examined, granular pericytes were localized in the perivascular spaces between the basal lamina of endothelial cells and the common basement membrane. Granular pericytes contained spherical electron-dense inclusion bodies with homogeneous matrices and lipid droplets. These inclusion bodies measured approximately 0.2–1.3 μm in diameter and were distributed throughout the cytoplasm. Granular pericytes gradually increased in size and number with time. However, they did not have pseudopodia. In capillaries in which filamentous and granular pericytes coexisted, the granular pericytes were consistently localized just outside the filamentous pericytes (Figure 1).

In the WKY, cytochemical studies revealed that the inclusion bodies of granular pericytes had acid phosphatase activity. In addition, in tracer experiments we demonstrated the reaction products of horseradish peroxidase in plasmalemmal vesicles of endothelial cells and occasionally in inclusion bodies of granular pericytes. Astrocytic end-feet surrounding the common basement membranes of the capillaries were well preserved in all WKY.

From each asymptomatic SHRSP we obtained at least 10 cerebral sections from the anteromedial and occipital cortices. Since these are the usual sites of stroke, morphologic studies revealed no abnormal lesions in SHRSP aged ≤8 weeks. Degeneration of
filamentous pericytes, characterized by decreases in the amount of cytoplasm and the numbers of filaments and cell organelles, was initially discovered in SHRSP aged 12 weeks. Filamentous pericytes became electron-dense and amorphous with time. After the degeneration of filamentous pericytes, focal swelling of the astrocytic end-feet appeared around the capillaries in asymptomatic SHRSP aged ≥16 weeks. Astrocytic matrices and filaments decreased in the end-feet with focal swelling although the mitochondria were well maintained (Figure 2). End-feet swelling was remarkable in deep layers of the gray matter and in the adjacent white matter although we did not examine deep areas of the white matter. In asymptomatic SHRSP aged >20 weeks, we discovered obvious attachment plaques in the swollen astrocytic cytoplasm adjacent to the common basement membranes of the capillaries. The attachment plaques were arrayed at almost regular intervals and were lined with numerous astrocytic filaments. Most filaments adhered to the plaques in the tangential direction (Figure 3). The number of cell organelles, especially rough endoplasmic reticulum and ribosomes, increased in swollen astrocytes that contained obvious attachment plaques. We occasionally observed fibrous astrocytes containing numerous astrocytic filaments and highly-developed nucleoli.

In asymptomatic SHRSP, in contrast to the degeneration of filamentous pericytes, granular pericytes increased in number and size. Granular pericytes were observed approximately 10 times as often in asymptomatic SHRSP aged 20 weeks as in WKY aged 20 weeks, were >5 μm wide and 20 μm long, and contained several kinds of inclusion bodies, some of which were membrane-bound while others consisted of small spherical globules and lipid droplets. Many granular pericytes had extensive smooth endoplasmic reticula. The number of inclusion bodies increased and they became vacuolated with age, although the amount of smooth endoplasmic reticulum decreased with time (Figure 4).

In asymptomatic SHRSP, cytochemical studies showed that the inclusion bodies and spherical globules were strongly positive for acid phosphatase activity. Tracer experiments revealed reaction products of horseradish peroxidase in transendothelial channels and vesicles of the capillary endothelium that had lost the protection provided by filamentous pericytes (Figure 5). Reaction products were also demonstrated in granular pericytes. Reaction products became more abundant in asymptomatic SHRSP aged >20 weeks. Endothelial tight junctions were intact in asymptomatic SHRSP.

The brains of all 30 SHRSP with symptoms of stroke were macroscopically swollen. We found 35 cerebral infarcts and 10 cerebral hemorrhages in these brains. We obtained at least 20 cerebral sections from the areas of infarction and hemorrhage. Morphologic studies showed that both astrocytes and astrocytic end-feet had become electron-lucent because of edema. These cells were composed of electron-lucent and amorphous cytoplasm, a few astrocytic filaments and granules, and well-maintained mitochondria. Astrocytes in these rats were in various phases of producing attachment plaques and astrocytic filaments. Some astrocytes contained much rough endoplasmic reticulum and ribosomes although they contained few filaments and only vague attachment plaques. Other astrocytes had obvious attachment plaques to which numerous filaments adhered. Some of these astrocytes were packed with innumerable astrocytic filaments (Figure 6). We demon-
stratified neuronal death around the capillaries surrounded by such fibrous astrocytes.

In SHRSP with symptoms of stroke, most filamentous pericytes were degenerated and consisted of atrophic cytoplasm and amorphous nuclei, much like those found in asymptomatic SHRSP. In contrast, granular pericytes were well-developed, morphologically active, and contained many cell organelles (dense bodies, ribosomes, endoplasmic reticula, and mitochondria). Granular pericytes and macrophages occasionally coexisted in the subendothelial spaces. Macrophages were also observed among the neural structures.

In SHRSP with symptoms of stroke, there was strong acid phosphatase activity in the inclusion bodies of granular pericytes. Reaction products of horseradish peroxidase were observed in transendothelial channels and vesicles of the endothelial cells and in granular pericytes. Reaction products were occasionally detected in the interendothelial junctions of capillaries tightly covered with fibrous astrocytes.

Characteristic findings of three groups are summarized in Table 1.

Cerebral capillaries in both WKY and asymptomatic SHRSP were variably and partially lined with a layer of pericytes. Results of the morphometric study are shown in Figure 7. At 12 weeks of age more than half of the capillaries had a pericyte lining ratio of 50–75% in both WKY and SHRSP. At 24 weeks of age the lining ratio in SHRSP decreased; 38% of the capillaries had a ratio of 0–25% (p < 0.01).

Discussion

It is well established that a barrier prevents an exchange of protein between the blood stream and the brain parenchyma. We suggested earlier that increased permeability of the periadventitial capillary endothelium must be considered in the pathogenetic mechanisms of cerebrovascular lesions.

We examined cerebral capillaries and found that they were covered with two kinds of pericytes: granular pericytes and filamentous pericytes. Our morphologic study revealed that the filamentous pericytes initially began to degenerate in SHRSP aged ≥12 weeks. Furthermore, our morphometric study demonstrated that the pericyte lining ratio in SHRSP was significantly less than that in WKY at 24 weeks of age. Our previous study demonstrated that cerebral blood flow decreased in SHRSP with the development of hypertension. We also discovered that deficiencies of nutrients and oxygen may cause medial necrosis in SHRSP. Recent publications have described the similarities in actins and myosins between pericytes and smooth muscle cells. Therefore, the degeneration of filamentous pericytes in SHRSP may be caused by reduced cerebral blood flow just as medial smooth muscle cells degenerate as blood flow decreases.

In one study, acute hypertension caused the blood–brain barrier to break down, whereas the blood–retinal barrier (which contains a higher density
FIGURE 4. Electron micrograph of cerebral capillary from stroke-prone spontaneously hypertensive rat without symptoms of stroke, aged 24 weeks. Capillary is covered with large granular pericytes (thin arrows) containing several kinds of inclusion bodies. Extensive smooth endoplasmic reticula are found within cytoplasm of granular pericyte. Swollen astrocytic end-feet (thick arrows) surround capillary as well as granular pericyte. L, lumen; EC, endothelial cell. Uranyl acetate and lead citrate stain.

FIGURE 5. Electron micrograph of cerebral capillary from stroke-prone spontaneously hypertensive rat without symptoms of stroke, aged 24 weeks. Abundant reaction products of horse-radish peroxidase are seen in endothelial channels and vesicles as well as in subendothelial space. L, lumen; EC, endothelial cell; SES, subendothelial space. Uranyl acetate stain.
TABLE 1. Characteristic Findings of Cerebral Pericytes and Astrocytes in WKY and SHRSP

<table>
<thead>
<tr>
<th>Finding</th>
<th>WKY</th>
<th>Asymptomatic</th>
<th>Stroke</th>
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<tbody>
<tr>
<td>Filamentous pericytes</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Granular pericytes</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Coexistence of both types of pericytes</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Increase in size and number of granular pericytes with age</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Degeneration of filamentous pericytes</td>
<td>−</td>
<td>+++, ++</td>
<td>+</td>
</tr>
<tr>
<td>Swelling of astrocytic end-feet</td>
<td>−</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Attachment plaque in astrocytes</td>
<td>−</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Increased number of astrocytic filaments</td>
<td>−</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Acid phosphatase activity in granular pericytes</td>
<td>+</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Horseradish peroxidase reaction products in granular pericytes</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Opening of interendothelial junction</td>
<td>−</td>
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WKY, Wistar-Kyoto rats; SHRSP, stroke-prone spontaneously hypertensive rats.

FIGURE 6. Electron micrograph of fibrous astrocyte from stroke-prone spontaneously hypertensive rat with symptoms of stroke, aged 28 weeks. Numerous astrocytic filaments and cell organelles, especially rough endoplasmic reticula and ribosomes, are seen within cytoplasm. Conspicuous nucleolus (NO) is observed in nucleus. Uranyl acetate and lead citrate stain.

of pericytes) remained intact and undamaged. Stewart et al.12 examined living, structurally normal brains from patients of various ages at biopsy. The biopsy specimens revealed that the thinning of capillary walls with aging was caused by a loss of pericytes. These authors suggested that the loss of pericytes resulted in less compensation for transient leaks of plasma components.12 We discovered focal swelling of astrocytes in SHRSP aged ≥16 weeks. Tracer experiments demonstrated reaction products of horseradish peroxidase in transendothelial channels of capillaries that were not lined with pericytes. Reaction products were also detected in granular pericytes around these lesions. Our results suggest that the degeneration of filamentous pericytes is intimately associated with permeability of the endothelium and leads to the swelling of astrocytic end-feet.

Numerous researchers have examined phagocytosis in the central nervous system and have described microglial, pericyte, and histiocyte responses under various conditions. Cancilla et al.13 reported on the role of pericytes in the uptake and digestion of exogenous protein after injury to the cerebral cortex brought about by freezing. Dodson et al.14 published a report of the pericytic response of cerebral tissue to ischemia. According to the observations of Mato et al.15 intraventricularly administered peroxidase and ferritin accumulated in granular perithelial cells and...
were present in only minute quantities in pericytes and microglial cells. We found small granular pericytes containing spherical inclusion bodies around bifurcations of the capillaries in WKY. In SHRSP granular pericytes contained several kinds of inclusion bodies. Intravenously injected horseradish peroxidase rapidly penetrated the endothelial cells, and reaction products were packed in the inclusion bodies of granular pericytes in SHRSP aged ≥20 weeks. Some inclusion bodies stained strongly positive for acid phosphatase activity. This activity showed the magnitude of leaks of plasma components in SHRSP. Granular pericytes became active and grew in size with the development of hypertension. They differed from macrophages in localization, shape, size, and cytoplasmic organelles including inclusion bodies, although they possibly served as scavenger cells in the central nervous system. In the brain of mammals such as humans, monkeys, cats, and rats a layer of astrocytic sheet-like processes can be found beneath the subpial basement membrane, and either an electron-dense layer or attachment plaques are commonly seen in the cytoplasm just beneath the plasma membrane abutting on the subpial basement membrane.16-18 In contrast to this, neither an electron-dense layer nor attachment plaques can be recognized in the cytoplasm of those processes surrounding the basement membrane of capillaries. Attachment plaques appeared just beneath the astrocytic plasma membranes adjacent to capillaries in SHRSP after astrocytic end-feet began to swell. Numerous bundles of astrocytic filaments adhered to the plaques, which were arrayed at almost regular intervals. Attachment plaques are special fine structures that have been found in the primary end-feet of astrocytes just beneath the subpial basement membrane.16-18 These plaques seem to play important roles, such as defense or material exchange, on the surface of the brain although the detailed mechanisms are unknown. We cannot define the factors that trigger the production of attachment plaques around capillaries. However, it is important to note that astrocytic swelling seems to accelerate the production of plaques. It has been reported19 that attachment plaques were clustered on the endothelial-facing but not on the lateral membranes of astrocytes, indicating that the cells are polarized. The functions of attachment plaques remain unclear, although it is possible that they are involved in ion transport. Neurons release K⁺ when they are active. Astrocytes have a high density of potassium channels on their end-feet,20 providing a possible route for the siphoning of K⁺ away from active neurons. Our study suggests that attachment plaques act as anchors to maintain the position of the swollen astrocytes and take part in the reconstruction of the swollen ones as well as the siphoning of K⁺. In other words, attachment plaques may help to maintain a stable neural environment. Formation of attachment plaques around capillaries should be considered as a positive defense reaction by astrocytes.

After the plaques became conspicuous, the numbers of both astrocytic filaments and cell organelles increased in the swollen cytoplasm. Swollen astrocytes developed into fibrous astrocytes packed with innumerable astrocytic filaments. We detected openings of the interendothelial junctions when endothelial cells were covered with fibrous astrocytes. Janzer and Raff21 demonstrated that astrocytes are responsible for inducing endothelial cells to form tight junctions. Our results suggest that fibrous astrocytes cannot adequately maintain tight junctions characteristic of the blood–brain barrier. As a result, the tight junctions become leaky.

We advance the following hypotheses: astrocytes perform the main function in the trophic interactions among endothelial cells, astrocytes, and neurons, and dysfunction of astrocytes disturbs the neural environment and results in neuronal death.

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KEY WORDS • endothelium, vascular • astrocytes • brain edema • rats
Ultrastructural changes in cerebral pericytes and astrocytes of stroke-prone spontaneously hypertensive rats.
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Stroke. 1990;21:1064-1071
doi: 10.1161/01.STR.21.7.1064

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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