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ARCHITECTURE OF THE MEDIAL SMOOTH MUSCLE OF THE ARTERIAL VESSELS IN THE NORMAL HUMAN BRAIN: A SCANNING ELECTRON-MICROSCOPIC STUDY

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Abstract

The architecture of the medial smooth muscle of arterial vessels in normal human brains was investigated using scanning electron microscopy. We could divide the arterial vessels into four subdivisions according to the number of the circular muscle cells. The arteries (>100 μm in diameter) had 4-20 layers of circular smooth muscle cells; individual circular muscle cells were spindle-shaped and occasionally had branches at their ends. Multidirectional muscle cells were observed in the medio-adventitial border only at the branching sites in large arteries (>300 μm), but at both branching and non-branching sites in the small arteries (100-300 μm). The nonterminal arterioles (30-100 μm) had 2-3 layers of circular muscle cells; most of the circular muscle cells had nodular or rod-like processes at their branched ends. Multidirectional muscle cells were most frequently observed in the medio-adventitial border in this subdivision at both branching and non-branching sites. The terminal arterioles (10-30 μm) had a single layer of circular muscle cells. Multipolar (stellate in appearance) smooth muscle cells were mainly seen in the medio-adventitial border at branching sites. The precapillary arterioles (7-10 μm) had a single layer of branched muscle cells; individual muscle cells had 2-4 circular branches on both sides of the central bulges.

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Key words: Cerebral arterial vessel, circular smooth muscle, human brain, multidirectional smooth muscle, multipolar smooth muscle, scanning electron microscopy.

Introduction

A study on the normal architecture of the media of cerebral arterial vessels is necessary for anatomical and physiological investigations of the vessels in various cerebrovascular disorders, because the media of arterial vessels has an important role in the regulation of cerebral blood flow. In this regard, scanning electron microscopy (SEM) can provide a broader view of the three-dimensional architecture of the media of arterial vessels than light microscopy or transmission electron microscopy (TEM). Miller et al. (1987) reviewed the methodologies that have been employed to allow examination of the vascular smooth muscle. However, vascular smooth muscle cells have not been well studied with SEM in the human brain. We (Shiraishi et al. 1986) have reported a method for removal of the adventitial connective tissue and used it to study the normal architecture of the medial smooth muscle of arterial vessels in dog brains using SEM. The three-dimensional architecture of the medial smooth muscle of the arterial vessels of the normal human brain was examined in the present investigation.

Materials and Methods

Arterial specimens were taken from non-affected parts of the brains of 12 patients under 70 years of age with head trauma or brain tumors, in whom lobectomy had been performed or autopsies carried out (Table 1). The smaller arterial vessel specimens were taken from the frontal lobe and those of the larger arterial vessels from the middle cerebral arteries and their major branches. For preparing the smaller arterial vessel specimens, pieces of the brain including small arteries, approximately 5×5×5 mm in size, were taken from the frontal lobe prior to fixation and the brain parenchyma was removed from the small arteries by ultrasonic waves, 20 kHz (SONIFIER, Model 200, Branson Sonic Power Company) in 0.1 M phosphate buffer for 1-2 min. These specimens were fixed in 1.5% glutaraldehyde. For preparing the larger arterial vessels, the middle cerebral arteries, left intact in the brain, were perfused with 3% glutaraldehyde and then

Table 1. Summary of the patients from whom the specimens were taken

Case no.	Age/Sex	Diagnosis	Specimen
1	45/M	glioblastoma	FL, MCA[a)]
2	60/M	glioblastoma	FL[b)]
3	60/M	glioblastoma	FL[b)]
4	38/M	contusion	FL, MCA[a)]
5	48/M	acute subdural hematoma	FL, MCA[a)]
6	21/M	contusion	FL, MCA[a)]
7	48/M	contusional hematoma	FL, MCA[b)]
8	17/M	contusion	FL, MCA[a)]
9	60/M	astrocytoma	FL, MCA[a)]
10	50/F	glioblastoma	FL[b)]
11	67/M	acute subdural hematoma	FL[b)]
12	37/M	astrocytoma	FL[b)]

MCA: middle cerebral artery
FL: frontal lobe
a) autopsy b) lobectomy

removed and further fixed in 1.5% glutaraldehyde. All the specimens were washed in 0.1 M phosphate buffer, and post-fixed with 2% OsO_4 for about 2 h at 40°C. For removal of the adventitial connective tissue, the specimens were washed repeatedly in distilled water, and treated with 8 N HCl for 20-40 min at 60°C until the media of vessels blackened with OsO_4 could be observed under an operation microscope (Desaki and Uehara 1981). The specimens were washed in distilled water, dehydrated in a graded ethanol series, immersed in isoamyl acetate, critical-point dried with CO_2, sputter-coated with platinum and observed under a Hitachi S-500 A scanning electron microscope.

In order to measure the number of smooth muscle layers, the transverse aspect of the vessels was observed. Individual muscle cells were easily isolated by separating the media of the vessels with a fine needle prior to coating (Fujiwara and Uehara 1982). This enabled accurate evaluation of the shape and dimension of the individual muscle cells in larger vessels (arteries and arterioles with the exception of terminal arterioles). For vessels smaller than terminal arterioles, the length of the smooth muscle cells were measured by tilting the specimen stage at the maximum angle along the long axis of the vessel.

Results

Using the modified method, the adventitial connective tissues were removed sufficiently from the human cerebral arteries smaller than 300 μm in diameter, but treatment with HCl for a longer time was necessary to remove the adventitial connective tissue in the cerebral arteries larger than 300 μm in diameter. In addition, the fixation of the vessels by perfusion with glutaraldehyde and reperfusion with whole blood used in experimental animals could not be adequately performed to maintain the continuity and the shape of the vessels prior to taking the specimens from the brains even in the autopsy cases. Disruption of the vessels occurred frequently in those smaller than precapillary arterioles during ultrasonication. Therefore, observations of the vessels smaller than precapillary arterioles or arteriovenous anastomosis were limited in the present study.

We could divide the human arterial vessels into four subdivisions on the basis of the number of the circular smooth muscle layers. The morphological findings were characteristic for each subdivision irrespective of the location and the individual from whom the vessels were taken.

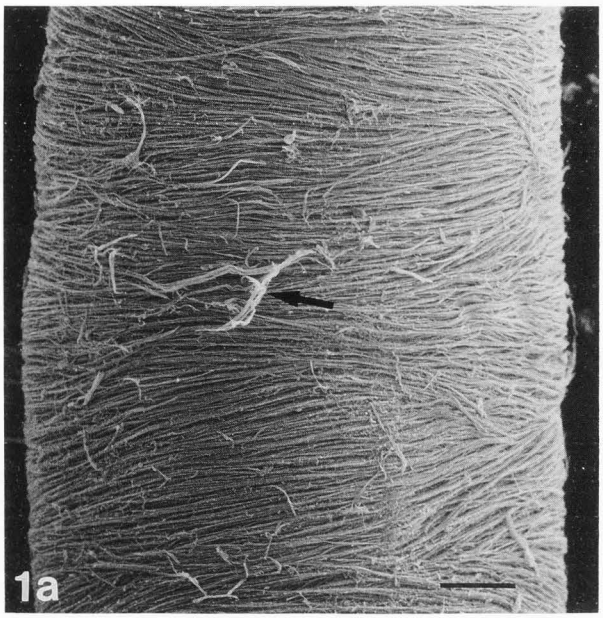
Arteries

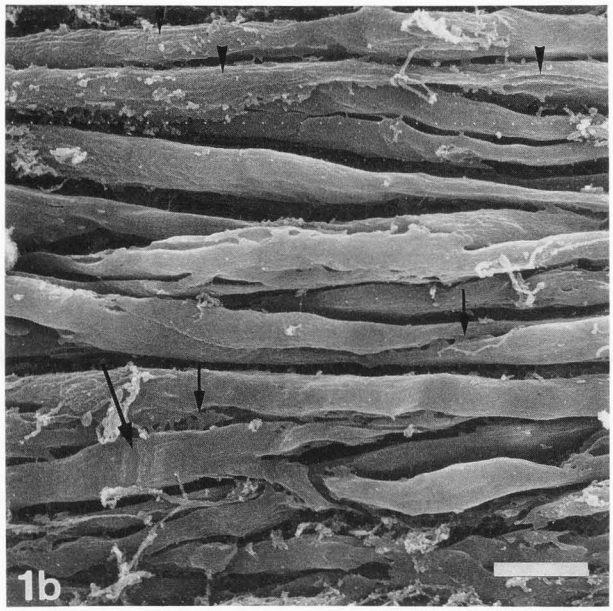
This subdivision included the middle cerebral arteries and their major pial and perforating branches larger than 100 μm in outer diameter. The architecture of the smooth muscle cells in these arteries consistently showed an ordered appearance with a circular arrangement (Fig. 1a). These arteries had 4-20 layers of circular smooth muscle cells.

On the adventitial surface of the outermost smooth muscle cells, longitudinal striations (fine furrows running parallel to the muscle cell axis) and diagonal or perpendicular indentations (shallow hollows which appeared to provide a pathway for adventitial connective fibers: this was supported by the observation of specimens containing remnants of adventitial tissue) were seen. Adjacent smooth muscle cells were connected to each other by many thin lateral process 0.1-0.5 μm in diameter (Fig. 1b).

Individual circular muscle cells were roughly spindle-shaped, and ranged from 75-100 μm in length and 2.5-6.0 μm in maximum width. The circular muscle cells occasionally had 2-3 short branches at both ends of the cells.

Multidirectional smooth muscle bundles (multidirectional bundles), were frequently observed in the medio-adventitial border of the arteries. In the larger arteries (>300 μm in diameter), the multidirectional bundles were almost always seen at branching sites where the vessels bifurcated or gave off large branches (Fig. 2). In the smaller arteries (100-300 μm), the multidirectional bundles were seen not only at branching sites but also at non-branching sites (Fig. 3a, b). Individual multidirectional muscle cells were also roughly spindle-shaped with short branches and had striations and indentations on their adventitial surface. Moreover, irregular-shaped cells with several short and long processes radiating from the cell body (multipolar cells), giving them a stellate-like appearance were often observed among the multidirectional bundles at branching sites of arteries (Fig. 3c). The adventitial surface of the multipolar





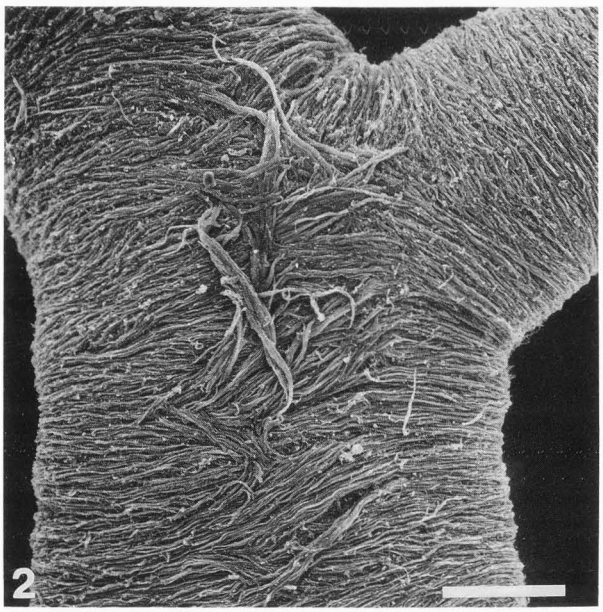


Fig. 1a,b The outer aspect of the media of the middle cerebral artery (about 830 μm in outer diameter).
a. Low magnification view shows an ordered appearance with a circular arrangement of smooth muscle cells. Smooth muscle cells partly peel off during the preparation (arrow). Bar=100 μm.
b. High magnification view shows striations (arrowheads) parallel with the long axis of the muscle cells and indentations (long arrow) diagonal or perpendicular to the long axis of the muscle cells on the adventitial surface of the outermost smooth muscle cells. Intercellular junctions with thin lateral processes (short arrows) are observed between adjacent smooth muscle cells. Bar=5 μm.

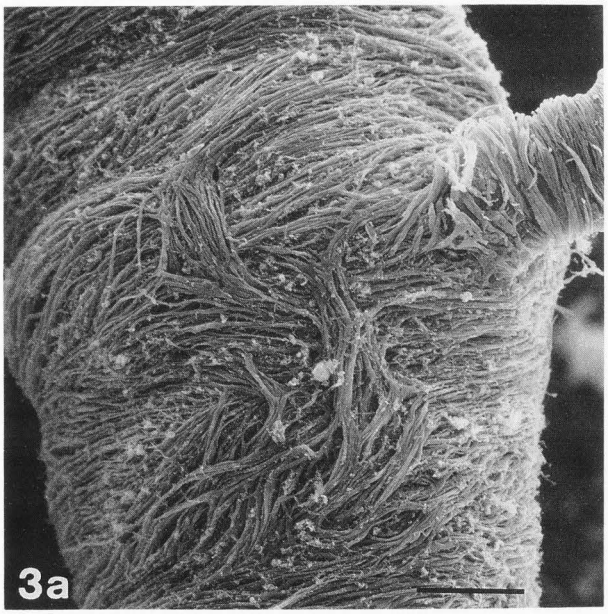
Fig. 2 Multidirectionally-oriented smooth muscle bundles (multidirectional muscle bundles) of the middle cerebral artery (about 450 μm in diameter). Bar=100 μm.

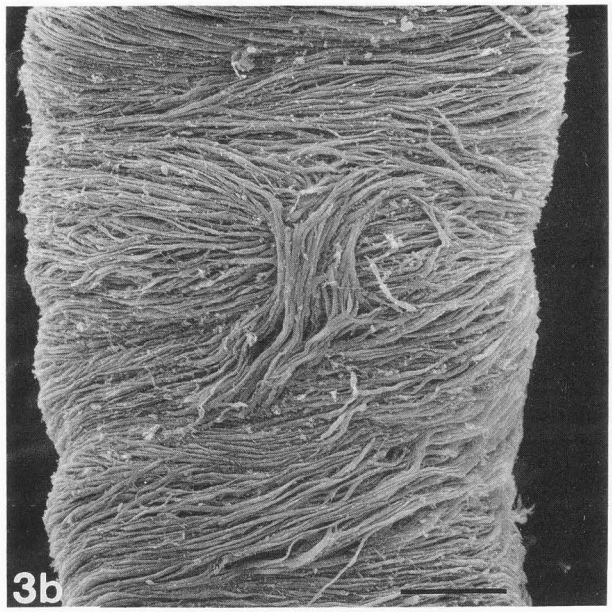
cells did not always clearly show longitudinal striations. However, multipolar cells were connected to multidirectional muscle cells and to underlying circular muscle cells by their processes. These multipolar cells possibly represent smooth muscle cells because of the intimate relationships between multipolar cells and other muscle cells. We, therefore, described multipolar cells as multipolar muscle cells below. Multidirectional muscle cells were also connected to each other and to the underlying circular muscle cells by their variously shaped processes.

Nonterminal arterioles

In the nonterminal arterioles with an outer diameter of less than 100 μm, the media consisted of 2-3 layers of circular muscle cells. The circular muscle cells were spindle-shaped with several short branches at both ends of the cells, and often had nodular or rod-like processes from the branched ends. Their length and maximum width ranged from 50-60 μm and 3-4 μm, respectively. The adventitial surface of the cells showed longitudinal striations.

Multidirectional muscle cells and multipolar muscle cells were most frequently encountered in the medio-adventitial border in the nonterminal arterioles compared with the other subdivisions of arterial vessels. They were distributed at both branching and non-branching sites. Multipolar muscle cells were almost always observed among multidirectional muscle cells, which displayed a reticular arrangement of the





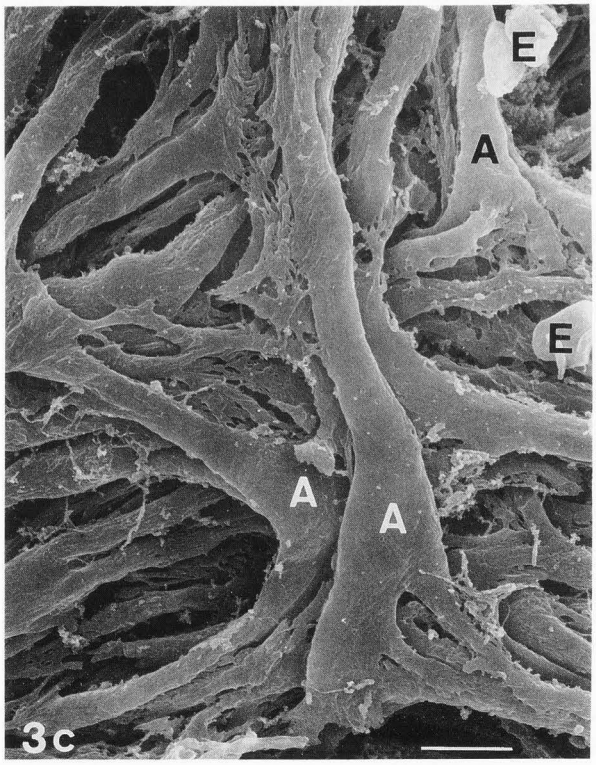


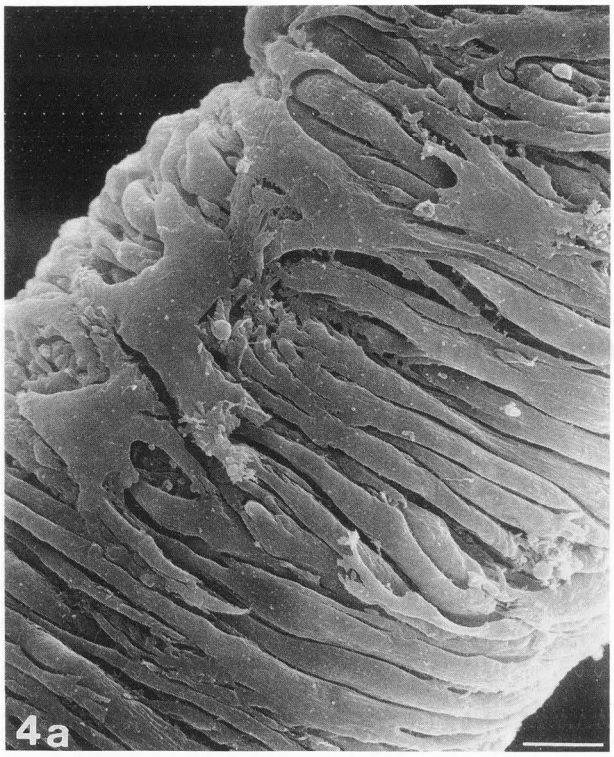
Fig. 3a-c Multidirectional muscle bundles of the small arteries.
a. Multidirectional muscle bundles at the branching site of the middles cerebral artery (about 230 μm in diameter). Bar= 50 μm.
b. Multidirectional muscle bundle at the non-branching site of a branch of the middle cerebral artery (about 240 μm in diameter). Bar=50 μm.
c. Irregularly shaped smooth muscle cells (A) (multipolar smooth muscle cells with a stellate appearance) among the multidirectional muscle bundles at the branching site. They are interconnected with each other and also connected to the underlying circular muscle cells by their processes. E: erythrocyte. Bar=5 μm.

medial surface but multipolar muscle cells were rarely observed to be grouped (Fig. 4a, b). Multidirectional muscle cells were connected to each other and to multipolar muscle cells by their variously shaped processes, and multidirectional and multipolar muscle cells were also connected to underlying circular muscle cells by their processes. The adventitial surface of the multidirectional muscle cells showed longitudinal striations, but the adventitial surface of the multipolar muscle cells did not always clearly show longitudinal striations.

Terminal arterioles

Terminal arterioles, ranging from 10-30 μm in diameter, consisted of a compact layer of spindle-shaped circular muscle cells. The circular muscle cells had nodular or rod-like processes at their branched ends. They were connected to each other in an "end-to-side" fashion by their nodular or rod-like processes and also in a "side by side" fashion by their thin lateral processes (Fig. 5). Some circular muscle cells had large bifurcations or trifurcations at one or both sides of the cells. Individual circular muscle cells ranged from 40 to 50 μm in length and 3.0 to 5.0 μm in maximum width. The adventitial surface of the cells showed longitudinal striations.

Multipolar muscle cells were frequently observed in the medio-adventitial border at branching sites and less frequently at non-branching sites (Fig. 6a, b).



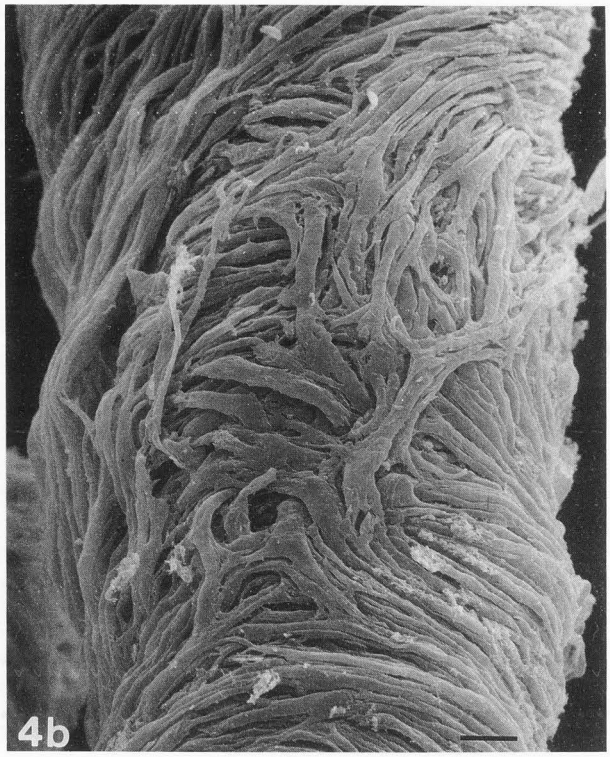


Fig. 4a,b Nonterminal arterioles in the frontal lobe. Vessels consist of 2-3 layers of spindle-shaped circular smooth muscle cells with nodular or rod-like processes from the branched ends.
a. A nonterminal arteriole about 48 μm in diameter. Several multipolar muscle cells can be seen to be grouped. Bar=5 μm.
b. A nonterminal arteriole about 85 μm in diameter. Multidirectional and multipolar muscle cells display a reticular arrangement. Bar=10 μm.

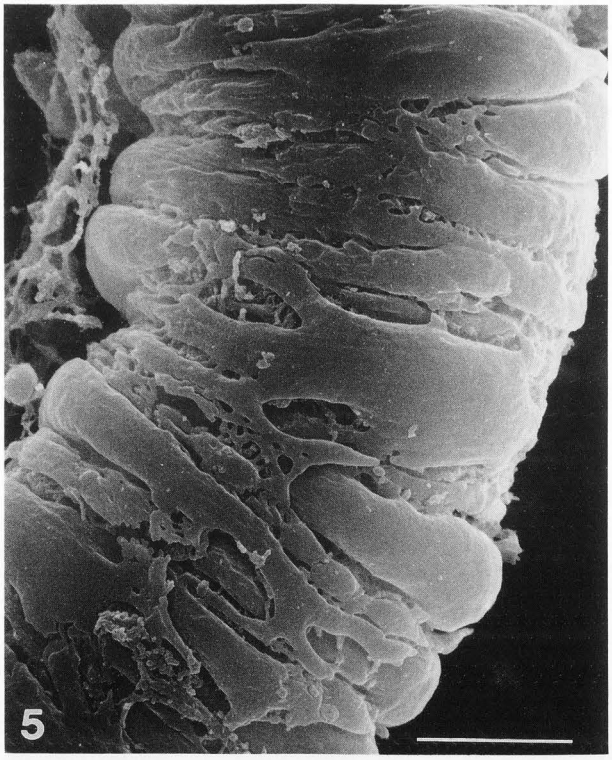
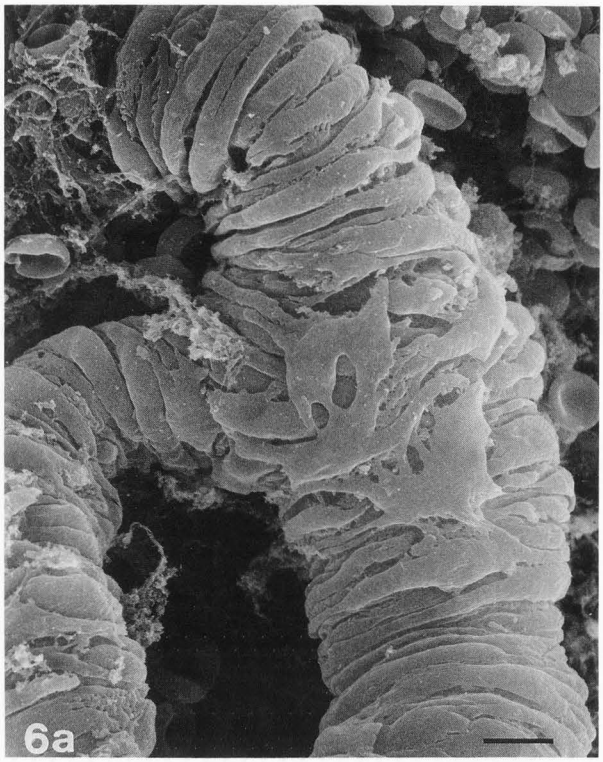


Fig. 5 A terminal arteriole (about 16 μm in diameter) in the frontal lobe consists of a single layer of spindle-shaped circular muscle cells with nodular or rod-like processes at their branched ends. Bar=5 μm.

They were attached to each other and to the underlying layer of circular muscle cells by their processes and did not always clearly show longitudinal striations on their adventitial surface.

Precapillary arterioles

Vessels with diameters of 7-10 μm consisted of a single layer of branched muscle cells. Individual branched muscle cells had bifurcations 2-3 μm in width on one or both sides of their central bulges, which was a characteristic morphological feature of the precapillary arterioles (Fig. 7a). The adventitial surface of the cells showed longitudinal striations. The bifurcated branches with many thin processes were also circularly oriented. As the vessels became smaller, the muscle cells acquired 3-4 branches on both sides of their central bulges and the cell-to-cell distance because wider up to 5 μm (Fig. 7b). The adventitial surface of the cells with 3-4 branches did not clearly show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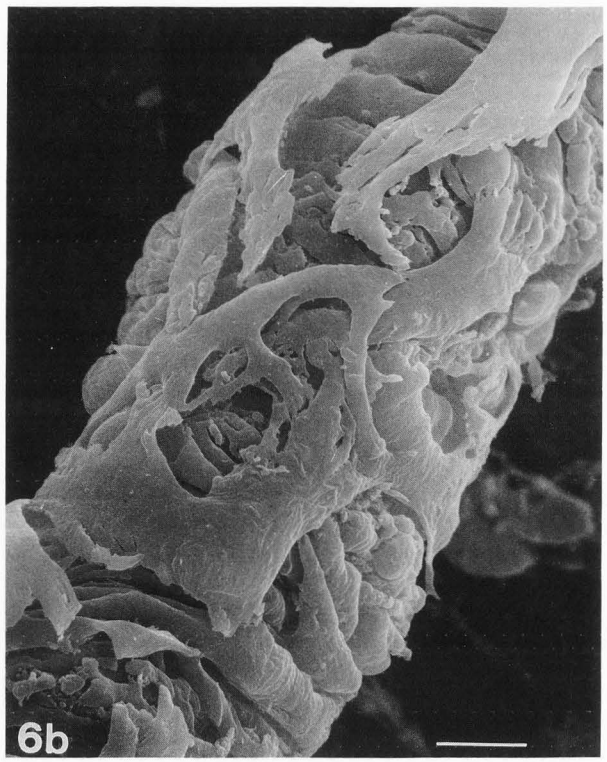
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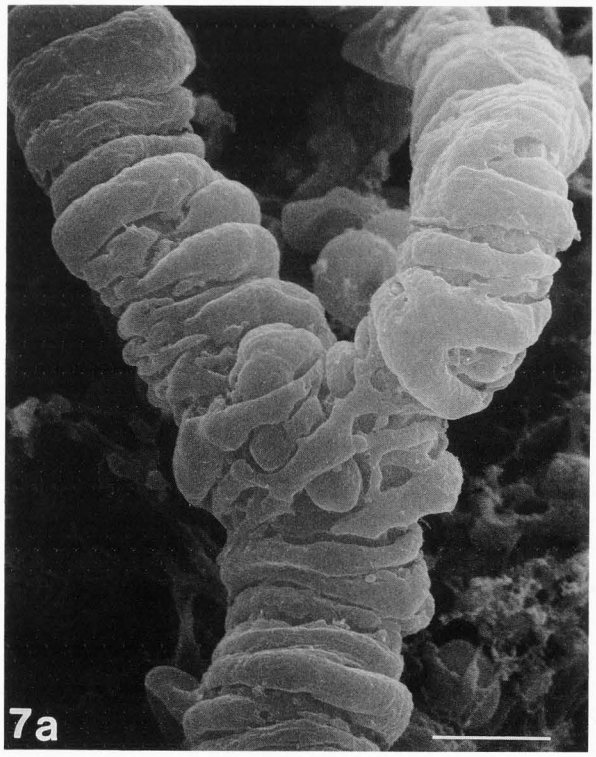
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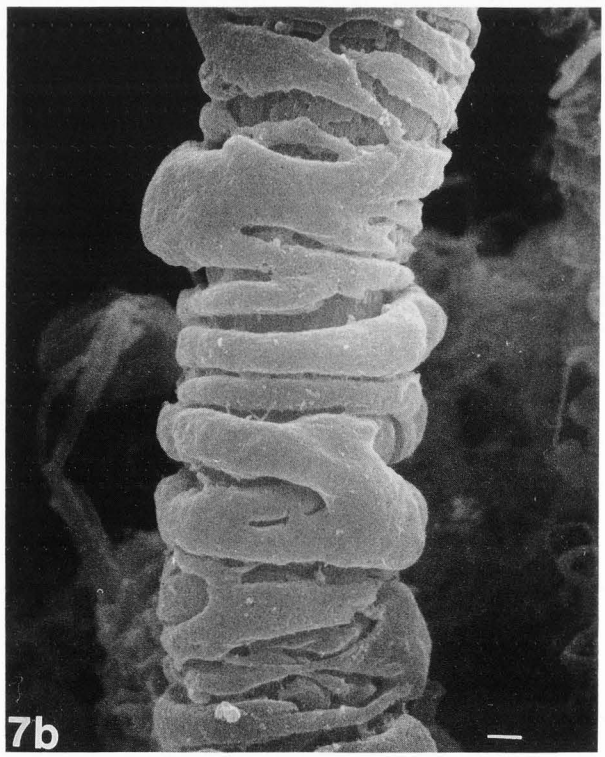
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longitudinal striations. The length of individual circular muscle cells ranged from 30 to 40 μm. No multidirectional or multipolar muscle cells were found at the branching or non-branching site. The vessels transformed into capillaries when the diameter of vessels became smaller than 5 μm.

Discussion

The specimen preparation technique used in the present study sufficiently removed the adventitial connective tissue from human cerebral arterial vessels, although it may have induced artifacts or distorted the cell morphology by ultrasonication or post mortem changes. In the control studies, we attempted ultrasonication in the vessels smaller than arterioles in the human brain, but failed to adequately remove the adventitial connective tissues. Next, we employed unfixed tissue that was first macerated by ultrasonication before immersion fixation. In addition, we attempted to take specimens from the brains as early as possible in autopsy cases. Despite rather drastic preparation techniques with ultrasonication or using specimens taken from autopsy brains, we obtained good preservation results of the cell surfaces of the vessels. In fact, the present results were comparable to the morphological findings of the specimens prepared without ultrasonication of the mesenteric arterial vessels in the monkey (Fujiwara and Uehara 1982) and of the cerebral cortical arterioles in the rat (Moore et al. 1985), and to those of the specimens treated with ultrasonication after fixation of the cerebral arterial vessels in the dog (Shiraishi et al. 1986).

In this study, we have arbitrarily classified the arterial vessels into four subdivisions according to the number of circular smooth muscle layers as observed in the dog brain. The term "arterioles" is generally used for vessels less than 100 μm in diameter, and "terminal arterioles" for vessels having only one complete layer of smooth muscle cells (Baez 1977). We have divided "arterioles" into "nonterminal arterioles" for vessels having 2-3 complete layers of smooth muscle cells and "terminal arterioles" for vessels having only one complete layer.

The architecture of the medial smooth muscle cells of cerebral arterial vessels in human brains was similar to that in dog brains. Strong (1938) described the helically oriented pattern of circular smooth muscle cells as a fundamental structure of muscular arteries by the microdissection methods. Cope and Roach (1975) reported using SEM that human cerebral arteries were composed of circularly-oriented smooth muscle cells at a pitch of 20-25 degrees. Hassler (1962) found by light microscopy that almost all muscle cells were arranged in a low pitch or nearly circular spirals. Walmsley (1983) also reported that the average pitch of smooth muscle did not deviate appreciably from a circular orientation. In the present study, the media was composed of circularly-oriented, spindle-shaped smooth muscle cells (circular muscle cells), having 4-20 layers in the arteries, 2-3 layers in the nonterminal arterioles, and a single layer in the terminal arterioles. As the vessels became smaller, the circular muscle cells gradually acquired variously shaped processes at their ends. The precapillary arterioles consisted of a single layer of branched smooth muscle cells with circularly-oriented large branches on both sides of their central bulges.

Characteristic morphological findings in the human cerebral arterial vessels as compared with those in canine vessels were: 1) Circular muscle cells occasionally had several short branches at their ends in the arteries and usually had short branches in the nonterminal arterioles and the terminal arterioles in humans, whereas these were characteristic findings in the terminal arterioles in dogs; 2) In the terminal arterioles, circular muscle cells occasionally had large bifurcations on one or both sides of their cell bodies in humans.

The longitudinal striations on the adventitial surface of smooth muscle cells have been revealed using TEM (Pease and Molinari 1960; Devine and Simpson 1971). Holley and Fahim (1983) observed similar features to grooves on the surface of arteriolar smooth muscle cells using SEM. They described that these depressed areas might correspond to zones near the cell surface where dense bodies were attached and have been pulled inward by contractile filaments. We also observed longitudinal striations on the adventitial surface of the multidirectional muscle cells as well as the circular muscle cells. These features were similar to those of smooth muscle cells of relaxed vessels in

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Fig. 6a,b Terminal arterioles in the frontal lobe.
a. Multipolar muscle cells are frequently observed at the branching site. (about 18 μm in diameter). Bar=5 μm.
b. Multipolar muscle cells at the non-branching site. (about 23 μm in diameter). They are interconnected with each other and connected to circular muscle cells by their variously shaped processes. Bar=5 μm.

Fig. 7a,b Precapillary arterioles.
a. This figure shows a portion of the terminal arteriole bifurcating into precapillary arterioles. The precapillary arterioles consist of a single layer of smooth muscle cells which have bifurcations on both sides of their central bulge. Bar=5 μm.
b. Small precapillary arteriole; circular muscle cells have many branches from their central bulges, and the cell-to-cell distance becomes wider. Bar=1 μm.

dog brains by perfusion fixation in our previous study (Shiraishi et al. 1986). These striations, however, were not always clearly observed on the surface of multipolar cells, suggesting an irregular arrangement or sparsity of membrane dense bodies in the multipolar cells.

We regarded multipolar cells as smooth muscle cells because of the close connections of their processes with other smooth muscle cells (circular and multidirectional muscle cells). However a combined study with TEM or other means such as fluorescence staining by using the actin probe nitrobenzoxadiazole-phallacidin (Barak et al. 1980) clarify the functional role of the multipolar cells.

Diagonal or perpendicular indentations on the adventitial surface of the outermost smooth muscle cells were frequently observed in arteries whereas in the vessels smaller than arterioles these indentations were not distinct. It is assumed that this difference is closely related to the thickness of the adventitial connective tissue.

The intercellular connection features using various kinds of processes are similar to those previously obtained by SEM of extracerebral vascular smooth muscle cells (Fujiwara and Uehara 1982; Holley and Fahim 1983). However, it is unclear from the present study which intercellular connection such as gap junctions, intermediate junctions or peg-and-socket junctions observed by TEM correspond to those by SEM.

We also found multidirectional and multipolar muscle cells in the medio-adventitial border widely distributed in the cerebral arterial vessels in human. These multidirectional and multipolar muscle cells were observed only at branching sites in large arteries (>300 μm), but at both branching and non-branching sites in small arteries (<300 μm) and nonterminal arterioles. There were multipolar muscle cells mainly at the branching sites in the terminal arterioles. We have regarded multipolar muscle cells as a kind of multidirectional muscle cell because of their distribution. Hassler (1962) observed longitudinal muscle cells only at branching sites of large cerebral arteries in the human and he speculated that the protrusion of the intimal cushions situated at the branching sites into the arterial lumen was regulated by the longitudinal muscle in and under the cushion. Walmsley et al. (1983) described the multilayered multidirectional orientation of smooth muscle cells near the bifurcations by fitting three cylindrical segments together. Our results indicate that multidirectional muscle cells are widely distributed from the arteries to the terminal arterioles not only at branching sites but also at non-branching sites. Regarding the functional significance of the multidirectional muscle cells, we consider that these cells may play an important role in strengthening the vessels against longitudinal (multidirectional) stress in addition to a regulatory function of cerebral blood flow, since the cerebral arterial vessels do not have discrete external elastic lamina or firm adventitial connective tissue.

In conclusion, we described the architecture of the medial smooth muscle cells of the cerebral arterial vessels in normal human brains using SEM. We will be able to compare these findings with those of pathological conditions.

References

Barak LS, Yocum RR, Nothnagel EA, Webb WW (1980) Fluorescence staining of the actin cytoskeleton in living cells with 7-nitobenz-2-oxa-1,3-diazole-phallacidin. Proc. Natl. Acad. Sci. USA 77, 980-984.

Baez S (1977) Microvascular terminology. In: Microcirculation. Kaley G, Altura BM (eds.) Vol. 1, Univ Park Press, Baltimore, pp 23-24.

Cope DA, Roach MR (1975) A scanning electron microscopic study of human cerebral arteries . Can. J. Physiol. 53, 651-659.

Devine CE, Simpson FO (1971) Surface features of smooth muscle cells from the mesenteric artery and vas deferens. J. Cell Sci. 8, 427-443.

Desaki J, Uehara Y (1981) The overall morphology of neuromuscular junction as revealed by scanning electron microscopy. J. Neurocytol. 10, 101-110.

Fujiwara T, Uehara Y (1982) Scanning electron microscopical study of vascular smooth muscle cells in the mesenteric vessels of the monkey: Arterial smooth muscle cells. Biomed. Res. 3, 649-658.

Hassler O (1962) Physiological intima cushions in the large cerebral arteries of young individuals. Acta Pathol. Microbiol. Immunol. Scand. 55, 19-27.

Holley JA, Fahim MA (1983) Scanning electron microscopy of mouse muscle microvasculature. Anat. Rec. 205, 109-117.

Miller BG, Evan AP, Bohlen HG (1987) Exposure of vascular smooth muscle cells for analysis with the scanning electron microscope. Scanning Microsc. 1, 1295-1313.

Moore SA, Bohlen HG, Miller BG, Evan AP (1985) Cellular and vessel wall morphology of cerebral cortical arterioles after short-term diabetes in adult rats. Blood Vessels 22, 265-277.

Pease DC, Molinari S (1960) Electron microscopy of muscular arteries; Pial vessels of the cat and monkey. J. Ultrastruct. Res. 3, 447-468.

Shiraishi T, Sakaki S, Uehara Y (1986) Architecture of the media of the arterial vessels in the dog brain: A scanning electron-microscopic study. Cell Tissue. Res. 243, 329-335.

Strong KC (1938) A study of the structure of the media of the distributing arteries by the method of microdissection. Anat. Rec. 72. 151-168.

Walmsley JG (1983) Vascular smooth muscle orientation in straight portions of human cerebral arteries. J. Microsc. 131, 361-375.

Walmsley JG, Campling MR, Chertkow HM

(1983) Interrelationships among wall structure, smooth muscle orientation, and contraction in human major cerebral arteries. Stroke 14, 781-790.

Discussion with Reviewers

JA Holley: One interesting possibility is that the isolated patches of longitudinal smooth muscles might represent locations where arterial branches had at one time occurred, but have since been reabsorbed. This hypothesis is testable. Did you notice any greater frequency of longitudinal muscle patches in arteries from older patients who presumably would have greater numbers of vascular reorganization events?
Authors: We are very interested in your hypothesis. We did not notice a greater frequency of longitudinal (or multidirectional) muscle patches in arterial vessels from non-affected parts of the brains in older patients. However, we found a greater frequency of multidirectional muscle cells and multipolar cells in patients with glioblastomas and arteriovenous malformations. Therefore, it is worth noting that multidirectional smooth muscle cells and/or multipolar cells are probably related to immature vessels or neovascularization.

JA Holley: The distribution of small diameter reticular processes, as seen in Figure 5, (which you call nodular or rod-like processes) appear to occur in discrete and infrequent areas. Is this true? And if so would you consider these likely areas for close communication between neural elements and the vascular system?
Authors: Nodular or rod-like processes were frequently observed in arterioles. We believe that these features indicate close communication between smooth muscle cells rather than between neural elements and the vascular system.

JG Walmsley: Would it be possible to observe the layers throughout the thickness of the media in order to make more general conclusion about the architecture of the medial smooth muscle? Attempts to peel away layers could damage the overall structure; how best could this observation be accomplished?
Authors: At present time, we do not know a proper method.

M Sjostrom: A comparative study of the organization of the arterial vessels from different parts of the brain, gray or white tissue, different nuclei etc. might be meaningful. This, above all, as the circulation is so selectively regulated in different parts of the brain and the architecture may be adapted accordingly. However, the authors do not consider this possibility at all. What is the author's comments on that?
Authors: In the present study, we observed arterial vessels taken from the areas distributed by middle cerebral arteries, but not from specific areas such as nuclei. However, there were no morphological differences in vascular smooth muscle cells in gray and white matter. We did not study the organization of the arterial vessels but the medial smooth muscle.

M Sjostrom: To what extent may shrinkage, or the technical procedure in other senses, be responsible for the specific appearances of subcellular structures at the different levels (the four subdivisions)?
Authors: The precise percentage of shrinkage is not known. We believe that our technical procedures do not produce specific influences of subcellular structures, because of the similarity of morphological features obtained with other SEM methods (Fujiwara and Uehara 1982; Moore et al. 1985; Shiraishi et al. 1986).

M Roach: Did you find any longitudinal muscle in the intima?
Authors: Yes, we did.