Microangioarchitecture of Rat Parietal Cortex
With Special Reference to Vascular "Sphincters"

Scanning Electron Microscopic and Dark Field Microscopic Study

KUNIO NAKAI, M.D., HARUMICHI IMAI, M.D., ICHIRO KAMEI, M.D.,
TORU ITAKURA, M.D., NORIHIKO KOMARI, M.D., HIROSHI KIMURA, M.D.,
TOSHIKABURO NAGAI, M.D., and TOSHIHIRO MAEDA, M.D.

SUMMARY Microangioarchitecture of the rat parietal cortex was studied by means of scanning electron microscopy and dark field microscopy. The richest supply of blood vessels in the parietal cortex was found in layer III + IV and layer V, where 2 isolated plexuses of microvessels were prominent. The appearance of the plexuses was quite different between motor and sensory areas. In the motor area the capillary plexuses were narrow and compact, while in sensory area the plexuses were wide and diffuse.

Characteristic ring formations, called ring-shaped-compressions in the present study, were frequently observed at branching sites of arterioles. The ring-shaped-compression probably corresponds to the precapillary sphincter. A similar structure was also seen in capillaries and venules and, therefore, it is likely that not only arterioles, but also capillaries and even venules, can actively change diameter to control cerebral blood flow.

BLOOD FLOW in the cerebral cortex changes under conditions such as cerebral thrombosis, intracranial hypertension and abnormal systemic blood pressure. It is important to understand the underlying mechanisms which control blood circulation within the cerebral cortex. There is a lack of morphological studies of small intracortical blood vessels which are responsible for cerebral microcirculation, although much has been known about extrinsic vessels and their neurogenic and metabolic reactions.

In the present study the basic differences of angioarchitecture of the somatomotor and somatosensory cortical areas have been studied by means of the scanning electron microscopy (SEM) and dark field microscopy. New information has been obtained about the active site in microvessels which constrict or dilate to control the cerebral microcirculation.

Materials and Methods

Twenty albino rats of the Wistar strain (200-250g) were used. The abdominal aorta and the inferior vena cava were cannulated with polyethylene catheters under sodium pentobarbital anesthesia (50 mg/kg, i.p.). The animals were then kept in an oxygen chamber filled with 95% O2 and 5% CO2. Concurrently with exsanguination from the inferior vena cava, perfluorochemical artificial blood (Fluosol-43, The Green Cross Corporation) was injected through the abdominal aorta. The PO2 and PCO2 were kept at 400-500 mm Hg and 35-40 mm Hg, respectively. When the total circulating blood was exchanged with Fluosol-43, the animal was perfused with 250 ml of mixed solution of 4% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 7 m at room temperature. After perfusion fixation, the whole body of the animal was immersed in the same solution for 2 days (postfixation). The materials were further processed for dark field microscopy and SEM, as described below.

1. Dark Field Microscopy

After fixation the brain was cut into coronal serial 80 μm thick sections with a vibratome. Serial sections were mounted on gelatine-coated glass slides and dried at room temperature. Sections were observed under a dark field microscope without dehydration and coverslipping.

2. Scanning Electron Microscopy

After fixation, 20 ml of polyester resin (Mercox CL2B, Dainippon Ink & Chemical Inc.) was injected via a catheter in the abdominal aorta at room temperature. Two hours later the brain was taken out and cut stereotactically into 4 mm thick coronal sections. The brain sections were kept in 20% NaOH solution for 2 days to lyse the tissue surrounding plastic casts. Small tissue plaques on the plastic casts were removed with an ultrasonic cleaner. The cleaned casts were freeze-dried for one day to preserve their fine structure, mounted on specimen mounts and covered with gold in an Ioncoater (Eiko 183). The gold-covered casts were observed under a scanning electron microscope (Hitachi S450) at 15 KV.

Results

1. Angioarchitecture of Rat Parietal Cortex

The cytoarchitecture of the rat parietal cortex has been studied in detail. The medial part of parietal cor-
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Figure 1. A schematic drawing showing the dorsal surface of rat parietal cortex. On the left, Krieg's cytoarchitectural mapping is shown in numbers (1-4). Hall's physiological mapping is shown on the right (m: motor, s: sensory area). Rectangles show our division of the parietal cortex. L (lateral) area refers to the motor cortex as determined by both physiological and cytoarchitectural studies. M (medial) area refers to the motor cortex determined by physiological study and mainly area 4 of cytoarchitectural studies. I (the intermediate) area refers to the sensory cortex determined by cytoarchitectural study but it is in the boundary between sensory and motor areas in physiological studies. The scale indicates Hall's A-P division of rat cerebral cortex. Arterial and venous systems are shown on the left and the right sides, respectively.

tex has an agranular appearance and the lateral part a granular appearance. We divided the parietal cortex into 3 areas according to Krieg's cytoarchitectural and Hall's physiological criteria (fig. 1). The medial (M) area was from a cortical region from 0.5 mm to 4.0 mm laterally from the midline. A section of parietal cortex between 5.0 mm and 8.0 mm was referred to the lateral (L) area. The area between M and L was called the intermediate (I) area. The observations were restricted to the areas between 0 and 1 in the anteroposterior plane from Hall's division and included the 3 areas mentioned above.

Most of the pial arteries which ran in the subarachnoid space of the parietal cortex were branches of the middle cerebral artery, although a small medial part was supplied by branches of the anterior cerebral artery. Pial arteries were approximately 50-150 μm in diameter. They ran parallel to the cortical surface in the subarachnoid space to give off many arterioles toward the surface at regular intervals of 100-150 μm. The arterioles which originated from the pial arteries perforated the cortical surface and ran perpendicularly toward the white matter (fig. 2a, b).

We classified the arterioles into 3 groups on the basis of the branching pattern and size of vessels (table). In Type 1, the size of arteriole was 30-40 μm in diameter and many small branches were found in the middle cortical laminae (layers III and IV), where small arterioles and capillaries formed complex networks of vessels as described below. This type of arteriole reached the deepest laminae (layer VI) of the cortex without changing its size throughout. Type 2 arterioles were slightly smaller (20-30 μm in diameter) in size and, characteristically, did not reach the deep cortical layers after producing branches of small arterioles and capillaries. Type 3 arterioles were 30 μm in diameter near the cortical surface. In the middle of the cortex, however, they suddenly reduced their size to 15 μm and reached the deep layers as small arterioles with some branches. Examples of

Table. Classification of arterioles. Schematic drawing shows 3 types of arterioles. Numbers in the columns indicate average numbers of each type of arterioles and venules per square mm counted in the medial (M) and lateral (I and L) areas. Only Type III arterioles have different distribution pattern between motor and sensory areas. Namely, M area has larger number of Type III arterioles than L area. M: medial area, I: intermediate area, L: lateral area.
these 3 types of arterioles are shown by 3 camera-lucida drawings in figure 3. They are also schematically illustrated in the table. The table summarizes the distribution pattern of these 3 types of arterioles in the 3 parietal areas. Medial (M) area and lateral (I and L) areas had almost equal numbers of Types 1 and 2 arterioles. Type 3 arterioles, however, showed a quite different distribution in different areas of the parietal cortex. Namely, the lateral parietal area (I and L area, granular cortex) had a larger number of Type 3 arterioles (6.3 per square mm) than the medial area (2.0 per square mm) (table).
The arterioles gave rise to many small arterioles and capillaries in all directions throughout their perpendicular course. A large number of capillaries and arterioles formed 2 prominent isolated plexuses which were obvious in the coronal sections (fig. 4). The upper band was seen through the lower part of layer III to layer IV and the lower band in layer V. The network of the 2 plexuses was quite different between the medial and lateral areas. In the medial area, both upper and lower capillary plexuses were narrower and more compact than in the lateral area. In the lateral area, in contrast, the plexuses were quite wide and diffuse and extended to the lower part of layer IV. In the intermediate area, the boundaries of plexuses became quite vague. Examination with the dark field microscope clearly showed the marked difference between the plexuses of the medial and lateral areas (fig. 4).

Small venules drained into relatively large venules of 40–100 μm in diameter. The large venules ran perpendicularly toward the cortical surface, as did the arterioles. Compared with the arterial system, the venous system showed quite different branching and distribution patterns (fig. 5). First, venules extended their branches at almost a right angle, while branching in the arterial system was at an obtuse angle. Second, small venules directly joined large venules. Third, large venules running toward the cortical surface were less in number than arterioles. Some venules were directed toward the white matter and joined there. The branching pattern of cortical venules was quite uniform throughout the parietal cortex. In the lateral parietal cortex, cortical perpendicular venules merged with pial veins, then Labbé's vein and, finally, with the lateral sinus. In the medial area, however, there were 2 draining pathways, one superficial and the other deep. The superficial draining pathway was composed of pial veins, ascending veins and the superior sagittal sinus. In the deep system, blood first drained into veins in the white matter, then the small vein of Galen, to the straight sinus and, finally, the lateral sinus.

2. Vessel Walls and Ring-Shaped-Compressions

The luminal surface of intracranial blood vessels was studied in corrosion casts by SEM and dark field microscopy. A pial artery was characterized by many prominent impressions formed by endothelial cell nuclei (fig. 6a). These impressions were ovoid and 30 μm by 10 μm in size. They occurred at regular intervals and were longitudinal to the long axis of blood vessels. They were conspicuous in the pial arteries but rarely found in the arterioles, capillaries and veins. It was quite easy to distinguish artery from vein in the subarachnoid space, because the pial vein had fewer ovoid impressions and a smoother luminal surface.

The arterioles, originating from pial arteries, showed a characteristic formation like that of a ring, called ring-shaped-compression in the present study. These were clearly observed under SEM and dark field microscopy (figs. 2a and 2b). A ring-shaped-compression was often seen at the branching sites of arterioles, especially in the relatively smaller arterioles, 15–30 μm in diameter (fig. 6b). As a rule, most small cerebral vessels, such as arterioles, capillaries and venules, often showed the ring-shaped-compressions but large cerebral vessels, such as the pial arteries and veins, lost these compressions as their size increased. Large capillaries of 10–15 μm in diameter and venules had fewer but distinct numbers of compressions compared with arterioles. Small capillaries less than 5 μm in diameter had fewer numbers of compressions than large capillaries. However, the compressions were sometimes seen even in small capillaries.

Discussion

There are critical drawbacks in the conventional dye method to study the angioarchitecture of the brain. For example, particles of a dye might not penetrate small capillaries. In the present study a simple, new method was adopted in which vibratome sections were observed with a dark field microscope.
FIGURE 5. Dark field microscopy of the parietal cortex. Ring-shaped-compressions are demonstrated in small arterioles and capillaries (arrows). The typical branching pattern of venule is seen; a venule has branches at almost right angles and small venules directly join a large venule. Bar indicates 50 μm.

without coverslipping. This method allowed preservation of all cerebreal blood vessels, even small capillaries with diameters of 5 μm. In air-dried sections, cerebreal vessels were easily identified and differentiated from the surrounding tissue because of the different light deflection by the former due to air filling the vessels. Concern about artifacts due to reduced blood in vessels during perfusion fixation was minimized by the use of artificial blood, Fluosol-43, during exsanguination before perfusion.

Observation of vibratome sections under the dark field microscope revealed 2 isolated bands forming a plexus of small vessels, mainly capillaries, in the parietal cortex. The upper band was in layer IV and the lower one, in layer V. These capillary plexuses were different in different areas of the parietal cortex. Different areas, e.g., the medial area (somatomotor) and the lateral area (somatosensory), showed different distribution patterns of capillaries. The somatomotor cortex showed a narrow and compact capillary plexus, while the somatosensory cortex showed a wider and more diffuse plexus. Schlesinger suggested that the richness of capillaries paralleled the number and size of cells in any given region of the cerebreal cortex with some exceptions. However, the results of the present study show that layer IV has the most prominent capillary supply. We, therefore, suggest that the density of blood vessels may not always parallel the number and size of nerve cells. We postulate that the number of synaptic connections and/or the metabolic rate of the area may correlate with the density of cerebreal vessels. This postulate may explain the recent observation of the lightest labeling of cells of layer IV by the 2-deoxyglucose method where the thalamic afferents terminate.

Anderson and Anderson observed a marked decrease in luminal diameter of precapillary arterioles with the appearance of a cone shape. They suggested that the cone shape was due to a precapillary sphincter. This study did not find such a decrease in diameter of arterioles under SEM but often observed characteristic ring-shaped-compressions in arterioles. It is likely that the ring-shaped-compression may indicate the presence of a precapillary sphincter because...
they are most often observed at branching sites of arterioles.

The precapillary sphincter could control flow rate by changing the diameter of these vessels. In the present study not only arterioles but also capillaries and even venules have ring-shaped-compressions, although their numbers were fewer in capillaries and venules than in arterioles. We have confirmed this ob-

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**Figure 6a.** Scanning electron micrograph showing pial vessels. Nuc
er identations (arrow) are prominent and regular in the pial artery (A). Bar indicates 50 μm. A: pial artery, V: pial vein.

**Figure 6b.** Replica of capillary and arteriole. Ring-shaped-compressions are seen at branching sites in capillary (C) arteriole (A) (arrows). Bar indicates 10 μm. Inset shows ring-shaped-compressions in dark field microscopy. The bar in the inset indicates 50 μm.
ervation in an unpublished study using transmission EM. Owman et al. demonstrated the presence of actin and myosin in cerebral capillaries by means of immunohistochemistry and we postulate that these proteins may be localized in ring-shaped-compressions of these vessels.

Previous authors demonstrated norepinephrine-containing nerve terminals in small intraparenchymal blood vessels including capillaries. Either stimulation of the locus coeruleus or the induction of lesions in these loci have been known to change cerebral blood flow of monkeys and cats.

In summary, results of this study suggest that in addition to arterioles, the capillaries and venules of the brain may actively change their diameter to control cerebral blood flow.

References

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