Neurobiology

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Microvasculature of the domestic ruminant brain: a vascular corrosion cast and immunocytochemical study

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Blood vessels of the brain in domestic ruminants (buffalo, cattle and sheep) were studied by vascular corrosion cast and immunocytochemical techniques at scanning electron microscopy (SEM).

Microvascular corrosion cast SEM

Nine heads (buffalo, cattle and sheep) were each perfused the maxillary artery with a physiological solution to wash the blood vessels. Next a methylmetacrylate mixture at low viscosity was injected, and after polymerization brains were soaked by 30% KOH solution for 1-2 weeks. Upon complete corrosion, casts were rinsed with distilled water, dried in a desiccator, mounted on stabs, coated with gold, and examined under SEM LEO 435 VP at 10 kV. The brain microvasculature of domestic ruminants, in general, showed morphological and structural features similar to these reported in other mammalian species.

Every cerebral hemisphere was supplied by terminal portion of the internal carotis (A. carotis interna), originated in a rete mirabile epidurale rostrale. From internal carotis originated:

- A. cerebri rostralis, directed rostro-medially, reached the cerebral longitudinal fissure, and supplies the hemisphere's medial face. By the rostral communicant artery communicates with the contralateral artery in correspondence with optic chiasma.
- A. cerebri media, supplied the hemisphere's lateral face by temporal, parietal and frontal branches.
- A. communicans caudalis, went caudally, anastomoses with contralateral artery and A. basilaris, and gave off the A. cerebri caudalis.

From final branches of the cerebral arteries (A. cerebri rostralis, A. cerebri media, and A. cerebri caudalis) originated arterioles that penetrated in perpendicular sense into cerebral cortex. These arterioles were the two types: shorts that supplied the cerebral cortex (Fig. 1a), and longs that supplied the white matter (Fig. 1b). Capillaries of the gray matter formed a thick network with some morpho-structural details (annular and ovoid formations). The capillary casts showed on the external surface ovoid formations single or double that represented the beginning of the neo-capillary. The neo-capillary proceeded in the direction of other neo-capillary that formed a normal capillary.

Immunogold-Labeling SEM Analysis

For the immunogold-labeling SEM analysis, the brains were cut into small fragments (rostral pole, caudal pole, and dorsal pars). Samples were immersed in PBS for 1hr, incubated for 1hr with a solution containing normal goat serum diluted 1:10 in PBS, and next with a primary polyclonal antibody directed toward CD133, diluted 1:1,500 in PBS, overnight at 4°C. After washing in PBS, the samples were incubated with gold-conjugated goat anti-rabbit IgG, diluted 1:200 in PBS for 1hr at room temperature. After washing in PBS, samples were fixed by 2.5% glutaraldehyde, and subjected to silver enhancement. Next samples were dehydrated, mounted on stubs, and examined under a LEO 435 VP at variable pressure in backscattered mode. Samples had not been coated by gold-palladium, so that the only conjugated gold deriving from the immunocytochemical reaction.

Samples showed the intense immunoreactivity on the external surface of the blood capillaries, located in the cerebral cortex (Fig. 1c). No immunoreactivity was observed in the cerebral cortex treated with PBS substituting the primary antibodies (negative control). These results display the presence the angiogenesis phenomena in the cerebral cortex of the domestic ruminant brain.

Figure 1.