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Choroid plexuses of the domestic ruminant brain: vascular corrosion cast, structural and immunocytochemical study

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The structural and functional features of choroid plexuses (CPs) in domestic ruminants (buffalo, cattle and sheep) were studied by light microscopy (LM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM). A. LM - CPs (lateral, III and IV ventricles), were immediately removed and cut in small pieces, washed in 0.1 M PBS, immersed in Tissue tech OCT compound, frozen in liquid nitrogen, sectioned by cryostat, and processed for NADPHd histochemistry. To tests NADPHd activity showed that choroid epithelial cells, and blood vessels contain an intense staining.

B. TEM - Heads were perfused through the maxillary artery with 0.1 M cacodilate buffer, pH 7.2, and then fixed with a mixture of this buffer and 2% glutaraldehyde. After 1hr, brains were removed, CPs collected were cut into minute fragments, post-fixed with 2% O_sO_4 for 2hr, dehydrated, and embedded in an EM bed of 812 resin. All embedded specimens were sliced into thin sections, stained with uranyl acetate and lead citrate, and examined under TEM at 40 kV. Transmission electron micrographs of the choroid epithelium show tight junction as well as intercellular spaces of variable extension on the surface of contact between two adjacent epithelial cells. Multiple cellular processes can be seen in cellular spaces. C. SEM – SEM analysis was performed on CP samples obtained by tree distinct procedures: (1) vascular corrosion cast technique, (2) intact tissue technique, and (3) immunogold-labeling SEM analysis. 1 - For the vascular corrosion cast technique, heads were perfused through the maxillary artery with a 0.9% physiological saline solution for cleaning of the vascular system. Next a methylmetacrylate mixture at low viscosity was injected, and after polymerization CPs 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. CPs microvasculature of domestic ruminants, in general, showed morphological and structural features similar to these reported in other mammalian species.

2 - For the intact tissue technique, heads were perfused trough the maxillary artery with 0.1 M phosphate buffered saline (PBS), pH 7.3, and fixed with Karnovsky's solution. After 12hr, CPs were removed, cut into small fragments, dehydrated in ethyl alcohol, and dried to the critical point. Some fragments, after the treatment with the fixative, were subjected to an ultrasonic treatment in a bath at 35kHz to avoid damage to tissue structures. This treatment latest 5 min for cleaning the apical surface of the choroid epithelium, and 15 min for detach the choroid epithelium from the basal membrane. The same treatment for 30 min allowed for the rupture of the epithelium basal membrane from deeper vessels. The specimens were mounted on stubs, and examined under a LEO 435 VP at 20 kV. The CPs samples subjected to sonication steps show the presence of cells with morphology distinct from their neighboring epithelial cells: extended cellular elements (Fig. 1a), and cells with many protrusions (Fig. 1b).

3 - For the immunogold-labeling SEM analysis CPs were immediately removed from the brain, incubated for 2 hr with a solution containing normal goat serum, diluted 1:10 in PBS and next with primary polyclonal antibodies (NOS I, Ang-2, VEGFR-3 and CD1133) overnight at 4°C. After washing in PBS, all samples were incubated with gold-conjugated goat ant-rabbit IgG, diluted 1:200 in PBS for 1 h at room temperature. After washings in PBS, samples were fixed by 2.5% glutaraldehyde in 0.1 M cacodylate buffer containing $CaCl_2$, pH 7.2, for 30 min. After fixation step and washings with distilled water, samples were subjected to silver enhancement process. Next, samples were dehydrated through an ethanol series, dried to the critical point, mounted in stubs, and examined at variable pressure in the backscattered electron mode. The localization of NOS I, presences of the Ang-2-positivity, VEGFR-3-positivity, and CD133-positivity, confirm that CPs in mammalian brain show a fundamental role for maintenance of the CNS microenvironment.

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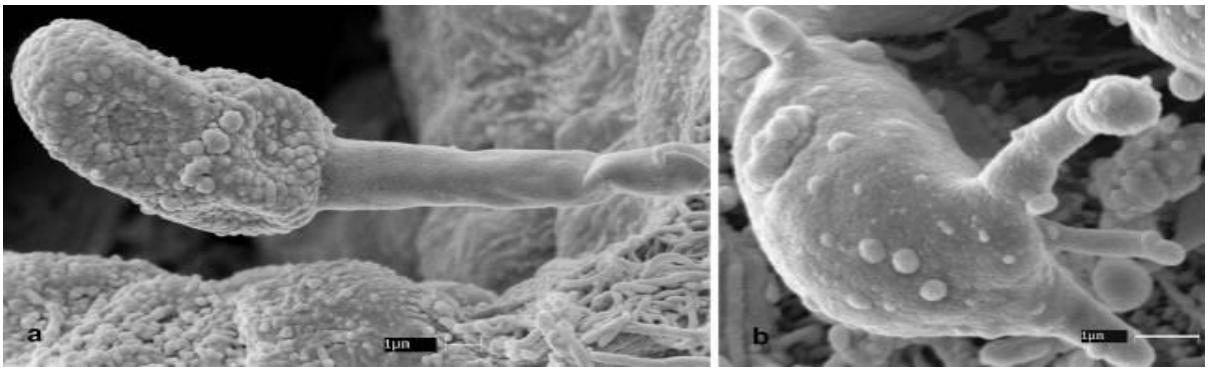

Figure 1. CPs of domestic ruminants. A: View of an extended cellular element of blood capillary. B: A cell with many protrusions.