



Ultrastructural organization of endothelial cells of the cerebellar cortex capillaries of the domestic bull

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ABSTRACT

The aim of this study was to characterize the ultrastructural organization of endothelial cells in the cerebellar cortex capillaries of the domestic bull. Investigation of cerebellar endotheliocytes is of considerable interest for both fundamental science and applied veterinary medicine and neurobiology, as these cells play a central role in regulating microcirculation and metabolism mechanisms. A clearer understanding of their ultrastructure may contribute to the development of new methods for diagnostics and treatment. Fragments of cerebellar cortex tissues were processed using standard methods, and were studied using electron microscopy. The terminology was described in accordance with the International Histological Nomenclature. The analysis demonstrated that the endotheliocytes of the cerebellar cortex capillaries of the domestic bull represent the primary structural component of the blood-brain barrier. They form the capillary lumen, are intercontacted by well-developed tight junctions, and rest upon a continuous basal membrane. The continuity of the latter and the presence of tight junctions restricts the possibility of spontaneous transport of macromolecular compounds from the bloodstream to the brain tissue.

Keywords

brain, cerebellar cortex, histohematic barriers, blood-brain barrier, homeostasis

Number of Figures: 4
Number of Tables: 0
Number of References: 23
Number of Pages: 6

Abbreviations

A : astrocyte;
AS : astrocyte stalk
BV : blood vessel lumen
CO : endothelial cell cytoplasmic outgrowths

EC : endothelial cell
P : pericyte
RBC : red blood cell

Introduction

The brain coordinates the functioning of all organs and systems of the body, ensuring its integrity and harmonious interaction with the environment. Its proper functioning depends on strict adherence to homeostatic principles [1,2]. The majority of metabolic processes that ensure the constancy of the environment occur between neural tissues and the vascular system at the level of the hemomicro-circulatory bed [3,4]. Within this system, hemocapillary, are the principal structural element, and their organization directly influences the physiological and biochemical processes of the central nervous system [5-7]. Based on these considerations, the objective of this study was to characterize the ultrastructural organization of endothelial cells in the capillaries of the cerebellar cortex of a domestic bull.

The ultrastructural investigation of endotheliocytes in the cerebellar cortex capillaries of domestic bulls (*Bos taurus*) holds considerable importance for both fundamental science and applied veterinary medicine and neurobiology. The relevance of this topic is determined by several key factors.

First, the cerebellum plays a critical role in motor coordination and balance in mammals, including bovines [8, 9]. Understanding the ultrastructural organization of capillary endothelial cells in the cerebellar cortex provides better insight into the mechanisms of microcirculation and metabolic support in this important part of the brain. Such knowledge may ultimately contribute to the development of new methods for diagnostics and treatment.

Second, capillary endotheliocytes play a key role in maintaining the blood-brain barrier, which protects the brain from toxins and pathogens [9-11]. Studying the ultrastructural characteristics of these cells in bovines expands our understanding of blood-brain barrier function across mammalian species. These findings also have relevance in the context of comparative neuroscience, where bovine data may serve as a useful model for broader interspecies extrapolation, including humans.

A final important aspect concerns the potential application of the research results in veterinary prac-

tice. Diseases associated with cerebellar microcirculation disorders can result in severe neurological disorders in bulls, negatively impacting their productivity and well-being. [9-11] Understanding the ultrastructural organization of endothelial cells provides a basis for the development of new methods for the diagnosis and treatment strategies for vascular and neurodegenerative diseases in bovines.

Results

Endothelial cells of the cerebellar cortex capillaries in the domestic bull constitute the primary structural component of the blood-brain barrier. These cells are flat, and elongated along the blood vessel. These cells contact each other with tight junctions and rest on a continuous basal membrane, thereby forming a capillary wall. Based on the obtained electronograms, the diameter of the lumen of the latter varied from 1.0 to 5.0 μm , while the thickness of the basal membrane varied from 40 to 200 nm. The continuity of the basal membrane, together with the presence of tight junction complexes, restricts the passage of macromolecular compounds from the bloodstream through the capillary wall into the brain tissue. The nuclei have an ovoid shape and occupy most of the cell, corresponding to its maximal height, which varies from 3.5 to 4.5 μm (Figure 1).

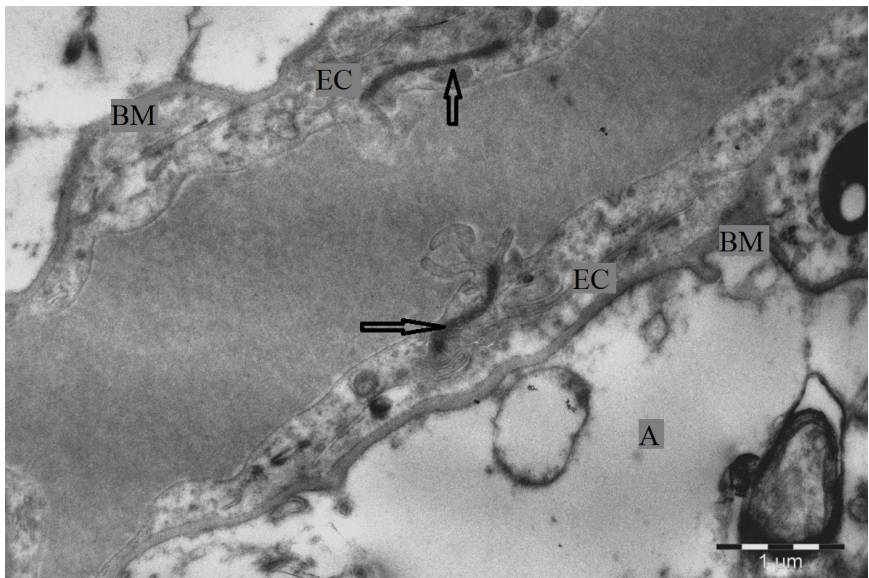


Figure 1. Ultrastructure of the basal membrane of the cortical capillary mammalian cerebellum. Electron micrograph: EC – endothelial cell; A – astrocyte; BM – basal membrane; ↑ – tight junctions.

In some cells, the the nuclear surface has a tortuous shape due to the undulations of the karyolem-

ma. (Figure 2) The nuclear envelope consists of two lipoprotein membranes, each 7 to 8 nm thick. The perinuclear space lies between the membranes. The membrane sometimes contains pores with diameters ranging from 70 to 100 nm. The central part of the matrix is usually electron light. Along the periphery of the nucleus, electron-dark accumulations of heterochromatin are noticeable, contacting the internal karyolemma.

The cytoplasm occupies the marginal part of the cell. Its thickness is slightly lower than the central part, the region occupied by nucleus, and varies within 1.3–1.8 μm . Electron-light mitochondria are detected in the cytoplasm. They contain thin cristae formed by folds of the inner membrane. The outer membrane of the organelles forms their shape, which varies from round to oval. The diameter of the mitochondria is

about 1.0 μm . Also the cytoplasm contained a significant number of ribosomes, which look like electron-dense granules with a diameter of 25 to 30 nm. In addition to individual ribosomes, polyribosomes, ribosomes clusters, as well as a small number of pinocytotic vesicles and single short channels of the granular endoplasmic reticulum were identified (figure 3).

The cytoplasmic membrane of endothelial cells formed their outer shell, and its thickness varies within 7.5–10.0 nm in the obtained electronograms. In accordance with the location of the endotheliocytes, it forms their luminal and basal surfaces. Luminal surface facing the inside of the vessel. With an increase in the functional activity of the endothelial cell, it is able to form outgrowths and protrusions. The basal surface of the cells contacts the basal membrane (Figure 4).

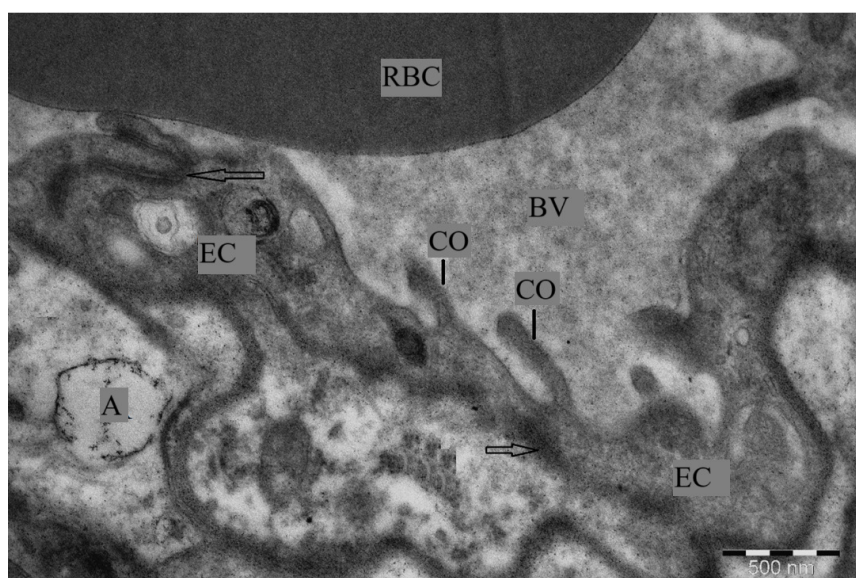


Figure 2.

Ultrastructure of the blood-brain barrier of the capillary of the cerebellar cortex of a domestic bull. Electron micrograph: EC- endothelial cell; P- pericyte; A- astrocyte; BV- blood vessel lumen; E- erythrocyte; $\uparrow$ - basal membrane.; PL- phagolysosomes; M- mitochondria.

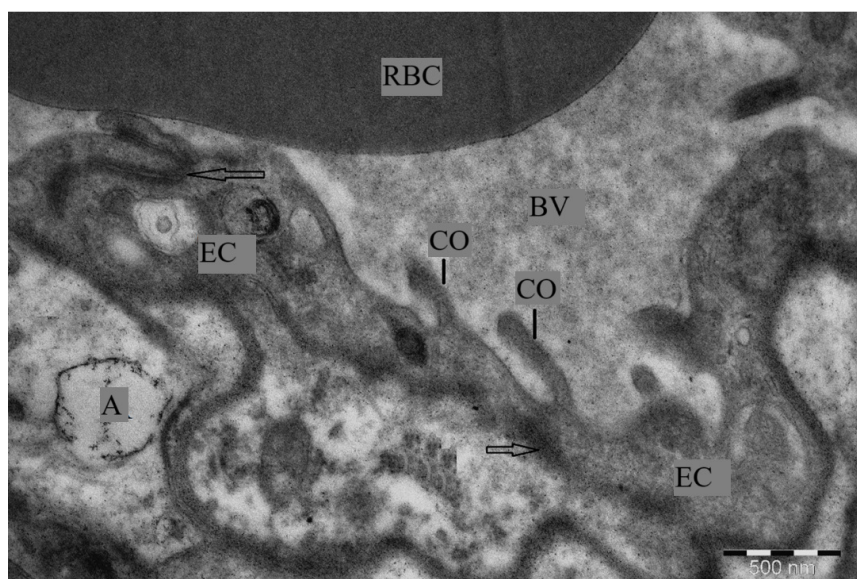
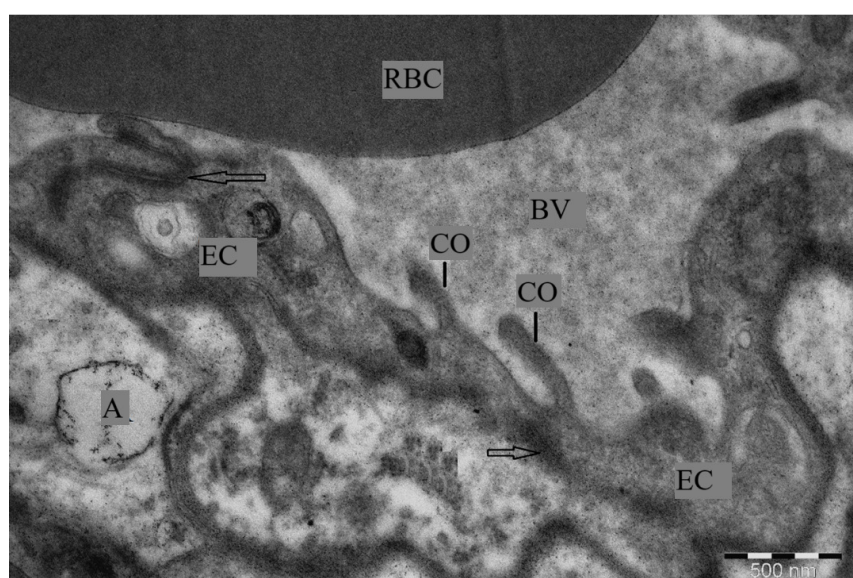


Figure 3.

Ultrastructure of the endothelial cell of the capillary of the cerebellar cortex of a domestic bull. Electron micrograph: EC - endothelial cell; P- pericyte; A- astrocyte; BV- blood vessel lumen; GER - granular endoplasmic reticulum; $\uparrow$ - basal membrane.

**Figure 4.**

Ultrastructure of a mammalian cerebellar cortex capillary endothelial cell in the active phase. Electron microphotography: EC—endothelial cell; A— astrocyte stalk; BV— blood vessel lumen; CO – endothelial cell cytoplasmic out-growths; RBC– red blood cell; ↑ – tight junctions.

Discussion

The present study revealed several key features and ultrastructural characteristics of endothelial cells that deserve detailed discussion.

First, the endothelial cells of the bovine cerebellar cortex capillaries exhibit a high mitochondrial density. This feature indicates an intensive level of metabolic activity, likely reflecting the high energy requirements of endothelial cells associated with transport of nutrients and oxygen to cerebellar neurons. [10, 15] Similar mitochondrial abundance has been reported of other mammalian species, emphasizing the universality of this phenomenon. [16-20]

Second, the obtained data indicate the presence of numerous pinocytotic vesicles within the endothelial cytoplasm. This indicates active transport of substances through the endothelium, which plays a key role in maintaining homeostasis of the cerebellar microenvironment. The variability in the number and size of pinocytotic vesicles may be influenced by the physiological state of the animal. [9,11,18]

The third important aspect is the presence of tight junctions between endotheliocytes, which form the blood-brain barrier. These junctions play a critical role in preventing potentially harmful substances from entering the brain from the blood [8,17]. The results show that tight junctions in the capillaries of the bovine cerebellar cortex are highly organized, which may be due to the need for strict control over the composition of the cerebellar microenvironment.

In addition, the presence of pericytes, which are closely associated with endothelial cells, is observed. Pericytes play an important role in regulating blood flow and maintaining the capillaries structural integrity [17]. Their presence in the capillaries of the bovine cerebellar cortex supports their critical role in

microcirculation dynamics in this part of the brain.

Thus, the results confirm that endothelial cells of the cerebellar cortex capillaries of the domestic bull are the primary element of the blood-brain barrier. Forming the lumen of the capillary, they contact each other with tight junctions and lie on a continuous basal membrane. The continuity of the latter and the presence of tight junctions block the possibility of spontaneous transport of macromolecular compounds from the bloodstream to the brain tissue.

CONCLUSION

In conclusion, this study highlights the complex ultrastructural organization of endothelial cells in the cerebellar cortical capillaries of the domestic bull. Revealed features, including high mitochondrial density, active pinocytosis, well-developed tight junctions, and the presence of pericytes, indicate the specialization and high functional activity of these cells.

Materials & Methods

Declaration of Generative AI and AI-assisted technologies in the writing process

AI-Assisted technologies were not applied at all stages of the work process.

Ethical Approval

The experiment was approved by the ethical committee Saint Petersburg State University of Veterinary Medicine.

Five fragments of the cerebellar cortex tissue from an adult domestic bull were used in the study. Each specimen measured no more than 2.0 mm³. The tissue samples were pre-fixed in a 2.0% glutaraldehyde solution prepared in a cacodylate buffer (pH 7.2–7.4) for 2 hours. Following primary fixation, fragments were rinsed in three changes of the same buffer. Post-fixation was performed in a 1.0% solution of osmium (VIII) oxide (diluted in cacodylate buffer, pH 7.2–7.4) for 1 hour. After post-fixation, the samples were dehydrated through a

graded series of ethanol solutions, and subsequently treated with absolute acetone. The dehydrated tissue fragments were then embedded in Epon-812 resin, in accordance with the generally accepted methodology [12]. From each embedded block, three ultrathin sections were obtained using an ultramicrotome (LKB-III, Sweden). The sections were contrasted with 2.0% aqueous uranyl acetate solution and then with lead citrate solution. Imaging was performed using a Jem-1011 electron microscope (JEOL, Japan) at magnifications ranging from 2500× to 3000× [13]. All terminology used in this study follow the International Histological Nomenclature [14].

Authors' Contributions

P.A.V. planned the experiments, carried out the experiment, contributed to the interpretation of the results. V.S.P. contributed to sample preparation, contributed to the interpretation of the results, took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Acknowledgements

The authors extend their sincere thanks to the members of Federal State Budgetary Educational Institution of Higher Education "Saint-Petersburg State University of Veterinary Medicine" for their help and support.

Conflict of interest

Authors declare that they have no conflict of interest. There were no ethical considerations to be considered in this research.

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How to cite this article

Prusakov AV, Vladimir S P. Ultrastructural organization of endothelial cells of the cerebellar cortex capillaries of the domestic bull. Iran J Vet Sci Technol.2026; 18(1): 71-76.
DOI: <https://doi.org/10.22067/ijvst.2025.88947.1399>
URL: https://ijvst.um.ac.ir/article_47458.html