Short communication UDC 616.8:[591.111:636.52
DOI:https://doi.org/10.46784/e-avm.v18i1.404

BLOOD-BRAIN BARRIER OF CAPILLARIES OF THE BRAINSTEM OF DOMESTIC CHICKEN

Alexey Prusakov[1*], Vladimir Ponamarev[1]

[1] Federal State Budgetary Educational Institution of Higher Education "Saint Petersburg State University of Veterinary Medicine", Saint Petersburg, Russia

Abstract

The aim of the study was to establish the structural organization of the blood-brain barrier of the brainstem capillaries of domestic chicken. The material used for the study was fragments of the brainstem tissue of the White Loman cross chicken. Their fixation and further processing to produce ultrathin sections were carried out in accordance with generally accepted methods. The obtained sections were examined using an electron microscope Jem-1011. The manuscript was written using the terminology of the International Histological Nomenclature. It was established that the brainstem blood-brain barrier of domestic chicken is formed by four structural elements: endothelial cells, basal membrane, pericytes, and processes of glial cells in astrocytes. The endotheliocytes that form the lumen of the capillaries are in contact using tight junctions, preventing the possibility of large molecular compounds to penetrate the blood-brain barrier. If there are pericytes accompanying the capillary, they are surrounded by cleavages of their basal membrane. The latter form a single layer along the capillaries, surrounding their wall from the outside. These structures ensure the selective transport of substances from the bloodstream to the nervous tissue.

Key words: neurology, brain, histohematic barriers, blood-brain barrier, homeostasis

[1*] Corresponding Author: psevdopyos@mail.ru

KRVNO-MNOŽDANA BARIJERA KAPILARA MOŽDANOG STABLA DOMAĆE KOKOŠKE

Aleksej Prusakov[1], Vladimir Ponamarev[1]

[1] Državna obrazovna ustanova visokog obrazovanja
"Sanktpeterburški državni univerzitet, Fakultet veterinarske medicine",
Sankt Peterburg, Rusija

Kratak sadržaj

Cilj ove studije je da se utvrdi struktura krvno-moždane barijere kapilara u moždanom stablu kod domaćih kokošaka. Za studiju su korišćeni fragmenti tkiva moždanog stable kokošaka hibridne rase "beli Loman". Njihovo fiksiranje i dalja obrada kako bi se dobili ultra tanki preseci obavljano je u skladu sa opšteprihvaćenim metodama. Dobijeni uzorci pregledani su pomoću elektronskog mikroskopa "Jem-1011". U radu je korišćena Internacionalna histološka nomenklatura. Ustanovljeno je da se krvno-moždana barijera moždanog stabla kod domaćih kokošaka sastoji iz četiri strukturalna elementa: endotelnih ćelija, bazalne membrane, pericita i produžetaka glijalnih ćelija u astrocitima. Endotelne ćelije koje formiraju lumen kapilara su povezane putem "*tight junctions*" veza, sprečavajući prolazak jedinjenja velike molekulske mase kroz krvno-moždanu barijeru. Ako periciti okružuju kapilare, oni su odvojeni pukotinom bazalne membrane. Površina bazalne membrane koja je okrenuta ka nervnom tkivu u kontaktu je sa izraštajima astrocita. Poslednje spomenuti formira jedan sloj duž kapilara, okružujući njihov zid sa spoljne strane. Ove strukture obezbeđuju selektivni transport supstanci iz krvotoka do nervnog tkiva.

Ključne reči: neurologija, mozak, histohematske barijere, krvno-moždana barijera, homeostaza

INTRODUCTION

Histohematic barriers ensure the constancy of the composition of tissue fluid by regulating metabolic processes between blood and tissues (Drozdova and Kundryukova, 2010; Gareev et al., 2023; Prusakova et al., 2024; Baltazar-Lara et al., 2022; Prusakova et al., 2023). In each organ, its structure is distinguished by its specific features (Akmalova et al., 2018). The main element of any histohematic barrier is the hemocapillary (Prusakov et al., 2019; Strizhikov et al, 2021). The pathogenesis of many diseases (Baryshev et al., 2022; Kuznetsov et al., 2022; Alekhin et al., 2023; Galitovskaya, 2018; Ponamarev et al., 2023), both in humans and animals, is based on structural changes in histohematic barriers (Yashin and Prusakov, 2021; Prusakov et al., 2020; Liu et al., 2023). Therefore, examining the characteristics of their organization is not only of theoretical interest but also critically important for practical veterinary medicine (Prusakov et al., 2019).

The study of the chicken blood-brain barrier involves routine histological examinations with hematoxylin and eosin staining. However, in the study, its ultrastructural organization was examined using electron microscopy. It enables the validation of hypotheses regarding the existence of specific intercellular junctions that are crucial for regulating barrier permeability. These data open new perspectives for understanding the mechanisms of protection of the central nervous system in birds and can be used to develop new methods for the treatment of diseases associated with impaired blood-brain barrier function. The data obtained from such studies are valuable and relevant, as they enhance our understanding of how substances are transported across histohematic barriers under both normal and pathological conditions.

The exchange of substances between the blood and the nervous tissue of the brain occurs through the blood-brain barrier. This barrier acts as a highly selective filter for various types of substances (Dyatlova et al., 2022).

Compared to mammals, the function of the blood-brain barrier in birds remains poorly understood. It is often assumed that their functions are alike due to the structural similarity between the avian and mammalian blood-brain barriers (Dunton et al., 2021).

Given the above, the goal was to establish the structural organization of the blood-brain barrier in the brainstem capillaries of the domestic chicken.

MATERIAL AND METHODS

The study material consisted of brainstem tissue fragments, each no larger than 2.0 mm^3, collected from five adult White Loman chickens that showed no signs of nervous system disease during their lifetime. At least five samples of brain stem tissue were collected from each animal. They were fixed in a 2.0% glutaraldehyde solution in cacodylate buffer (pH 7.2–7.4) for 2 hours. Then, after washing in three portions of the same buffer, they were postfixed in a 1.0% osmium tetroxide solution (prepared in cacodylate buffer, pH 7.2–7.4) for 1 hour. After that, the samples were dehydrated in ascending alcohols and absolute acetone. Then they were poured into Epon-812 in accordance with the generally accepted method (Anwar Ul-Hamid, 2018). Ultrathin sections were obtained on an ultramicrotome (LKB-III, Sweden), contrasted with a 2.0% aqueous solution of uranyl acetate and a solution of lead citrate (Reynolds, 1963). The resulting ultrathin sections were photographed using a Jem-1011 electron microscope (JEOL, Japan) at magnifications of 2500–3000. The manuscript was prepared using terminology from the International Histological Nomenclature (Semchenko et al., 1999). The experiment was approved by the ethical committee Saint Petersburg State University of Veterinary Medicine.

RESULTS AND DISCUSSION

It was established that the blood-brain barrier in the brainstem of the domestic chicken functions as a boundary structure separating nervous tissue from the circulatory system. Four structural elements are clearly distinguishable in their composition: endothelial cells, basal membrane, pericytes, and processes of glial cells in astrocytes. (Figure 1)

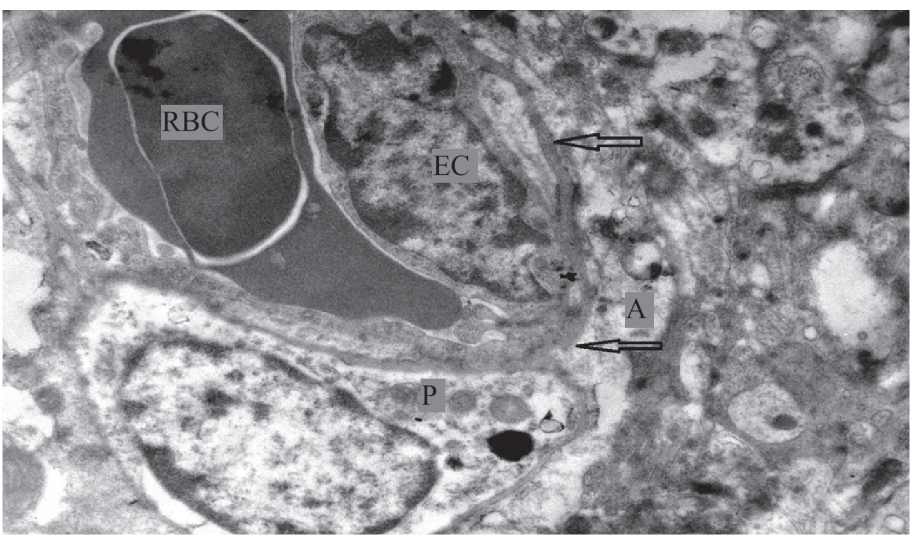

Figure 1. Ultrastructure of the blood-brain barrier of a bird's brainstem capillary. Electron microphotography (magnification × 15000): EC—endothelial cell; RBC—red blood cell in the lumen of a blood vessel; A – astrocyte stalk; P—pericyte; ↑ – basal membrane

Endothelial cells form a continuous lining of the capillaries that traverse the brainstem's nervous tissue, connecting to one another via tight junctions. These junctions effectively prevent the spontaneous passage of large-molecular compounds across the blood-brain barrier. (Figure 2)

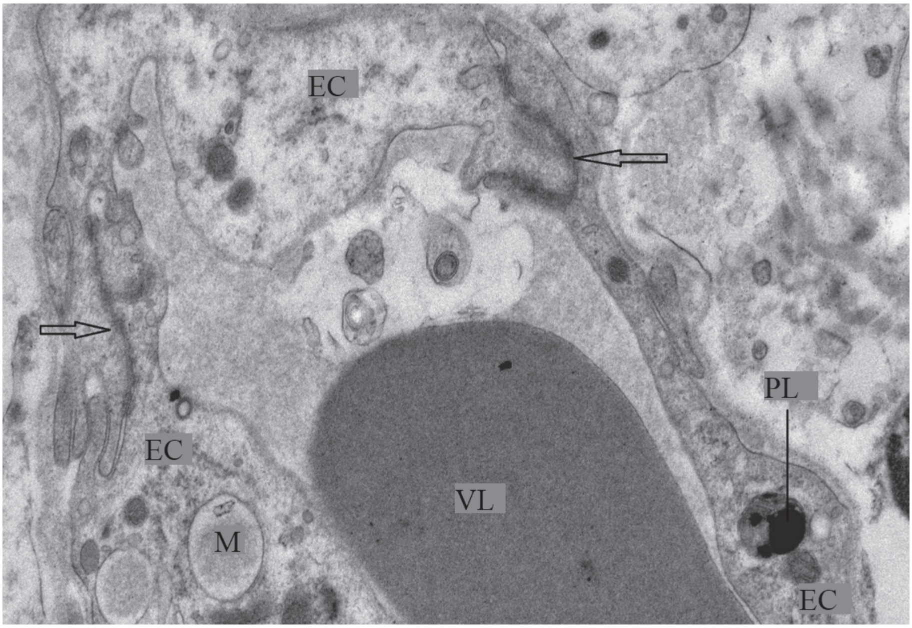

Figure 2. Ultrastructure of an endotheliocyte in a bird's brainstem capillary. Electron microphotography (magnification × 15000): EC - endothelial cell; VL – vessel lumen; M – mitochondria; PL – phagolysosome; ↑—basal membrane

Each endotheliocyte contains one nucleus, oval or elongated. The nuclear matrix in the central part of the nucleus is light, and heterochromatin in the form of electron-dense lumps is concentrated along the inner layer of the karyolemma. The nuclei occupy a greater volume of cells, while the cytoplasm surrounds them in the form of a thin rim. Despite its small volume, the cytoplasm of endothelial cells is rich in organelles, primarily various electron-dense ribosomes and polyribosomes. Additionally, it contains occasional short cisternae of rough endoplasmic reticulum, as well as isolated lysosomes and phagolysosomes. There are also mitochondria that have an electron-bright mitochondrial matrix. Owing to their low electron density, the mitochondria display sparse but distinguishable dark cristae - folds of the inner mitochondrial membrane. Occasionally, channels of the lamellar Golgi complex are also observed.

The electron diffraction patterns studied did not show any signs of active pinacytosis. This was evidenced by a small number of pinocytotic vesicles in the cytoplasm of endothelial cells.

In accordance with the location of the endothelial cells of the brainstem capillaries, two surfaces are distinguished: luminal and basal. The luminal surface faces the capillary lumen. Depending on the functional state of the cell, its topography can change. Thus, with increased activity, it can form many outgrowths and protrusions. The basal surface contacts the basal membrane.

The basal membrane is a non-cellular structure formed by a layer of fibrillar material. Its thickness varies between 40 and 100 nm. With the side facing the nervous tissue, the basal membrane contacts either the processes of astrocytic glia cells or the accompanying cells, pericytes (Figures 1 and 3). In areas where pericytes are absent, the blood-brain barrier's thinnest sections are observed, along with noticeable loosening. However, where pericytes are present, the basal membrane splits, forming duplications that entirely encase their bodies.

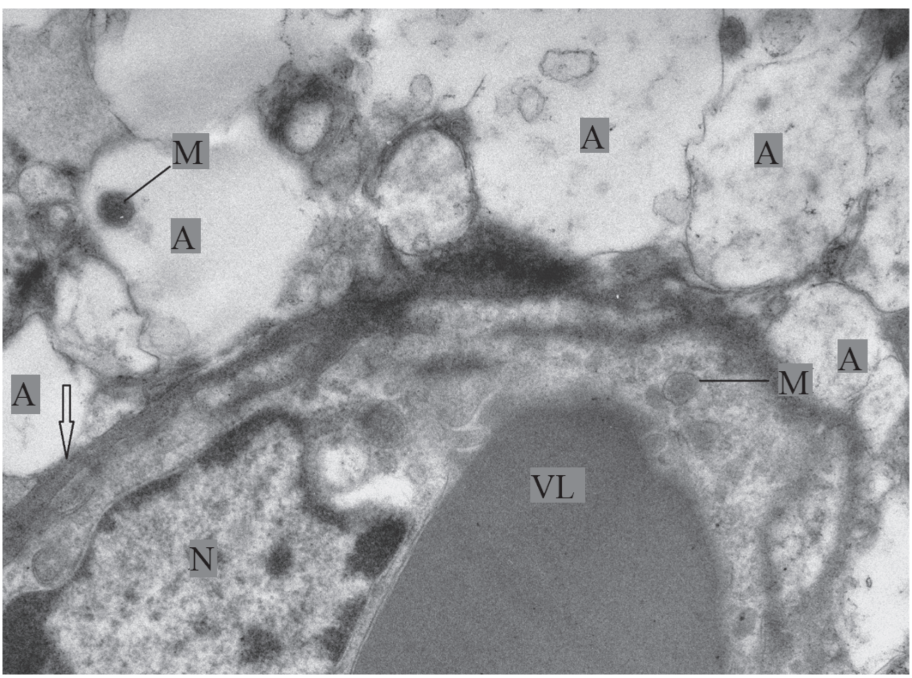

Figure 3. Ultrafine links between the avian brainstem capillary endotheliocyte and astrocytes. Electron micrography (magnification ×15000): A – astrocyte stalks; N – endothelial cell nucleus; VL – vessel lumen; M – mitochondria; ↑—basal membrane.

Pericytes protrude the cells enveloped in a basal membrane. Their cytoplasm is poor in organelles. It contains single mitochondria with a light matrix and small electron-dense lysosomes. Additionally, a few channels of the granular endoplasmic reticulum, small heterogeneous granules, and phagolysosomes are also present. Pericyte nuclei have a rounded-elongated shape with an electron-light matrix. In the phase of increased functional load, changes in pericytes, in the shape and size of the nuclei are noted. Thus, they increase in size, and their nuclear membrane becomes convoluted. In this case, large, dark heterogeneous phagolysosomes are revealed in the cytoplasm of these cells.

Pericyte-free areas of the basal membrane, as well as the surrounding basal membrane, are in contact with the processes of astrocytes. The ends of the latter are expanded, and thin microfilaments and a small number of mitochondria are detected in their cytoplasm. Due to the processes of astrocytes along the capillaries, a single layer is formed, surrounding their wall from the outside. (Figure 3)

CONCLUSION

Thus, the blood-brain barrier of the brainstem in the domestic chicken is composed of four structural elements: endothelial cells, the basal membrane, pericytes, and the processes of glial cells in astrocytes. Endothelial cells forming the lumen of capillaries contact by means of tight contacts, preventing the possibility of penetration of large-molecular compounds through the hematoencephalic barrier. If there are pericytes accompanying the capillary, they are surrounded by splits in their basal membrane. The surface of the basal membrane facing the nervous tissue is in contact with the processes of astrocytes. Along the length of the capillaries, a single layer of astrocyte processes surrounds the capillary wall externally. These structures provide selective transport of substances from the bloodstream to the nervous tissue.

ACKNOWLEDGEMENT

Author's Contribution:

AP – Research design, collecting samples, literature review, results analysis;
VP – Writing of the manuscript, translation, literature review, results analysis.

Competing interest

The authors declare that they have no competing interests.

REFERENCES

Akmalova G.M., Ron G.I., Musina L.A., Chernysheva N.D. 2018. Morphological features of the structure of elements of histohematic barriers in diseases of the oral mucosa. In: *Proceedings of the thirteenth Siberian Congress: "Dentistry and Maxillofacial Surgery with International Participation"*; All-Russian Symposium: "New Technologies in Dentistry", 20-23.

Alekhin Yu.N., Popova O.S., Ponomarev V.S., Parshin P.A. 2023. The effect of farnesoid X receptor agonist on postprandial lipemia in rats receiving a diet containing a supraphysiological dose of fats. Development and registration of drugs, 12(2): 174-184. https://doi.org/10.33380/2305-2066-2023-12-2-174-184

Anwar Ul-Hamid. 2018. A Beginners' Guide to Scanning Electron Microscopy. Switzerland: Springer Cham. https://doi.org/10.1007/978-3-319-98482-7

Baltazar-Lara R., Zenil J.M., Carranza M., Ávila-Mendoza J., Martínez-Moreno C.G., Arámburo C., Luna M. 2022. Growth Hormone (GH) Crosses the Blood-Brain Barrier (BBB) and Induces Neuroprotective Effects in the Embryonic Chicken Cerebellum after a Hypoxic Injury. International Journal of Molecular Sciences, 23(19): 11546. https://doi.org/10.3390/ijms231911546

Baryshev V.A., Popova O.S., Ponamarev V.S. 2022. New methods for detoxification of heavy metals and mycotoxins in dairy cows. Online Journal of Animal and Feed Research, 12(2): 81-88. https://doi.org/10.51227/ojafr.2022.11

Drozdova L.I., Kundryukova U.I. 2010. Poultry liver – a living laboratory for assessing the quality of feeding and maintenance. Agrarian Bulletin of the Urals, 5(71): 68-70. \

Dunton A.D., Göpel T., Burggren W., Ho D.H. 2021. Form and function of the vertebrate and invertebrate blood-brain barriers. International Journal of Molecular Sciences, 22(22): 12111. https://doi.org/10.3390/ijms222212111

Dyatlova A.S., Novikova N.S., Yushkov B.G., Korneva E.A., Chereshnev V.A. 2022. The blood-brain barrier in neuroimmune interactions and the development of pathological processes. Herald of the Russian Academy of Sciences, 92(10): 950-960. https://doi.org/10.1134/S1019331622050100

Galitovskaya N.V. 2018. Anatomical characteristics of the brain in poultry / N. V. Galitovskaya. In *Current issues in veterinary science and biotechnology: ideas of young researchers: Proceedings of the student scientific conference*, Chelyabinsk, Russia. 80-86.

Gareev K., Tagaeva R., Bobkov D., Yudintceva N., Goncharova D., Combs S.E., Ten A., Samochernych K., Shevtsov M. 2023. Passing of Nanocarriers across the Histohematic Barriers: Current Approaches for Tumor Theranostics. Nanomaterials (Basel), 13(7): 1140. https://doi.org/10.3390/nano13071140

Kuznetsov Yu.E., Lunegov A.M., Ponomarev V.S., Romashova E.B. 2022. Correlation relationships between the content of total bile acids and the main biochemical parameters of blood in minks (Mustela vison Schreber, 1777). Agricultural biology, 57(6): 1217-1224. https://doi.org/10.15389/agrobiology.2022.6.1217rus

Liu H., Lin H., Xu T., Shi X., Yao Y., Khoso P.A., Jiang Z., Xu S. 2023. New insights into brain injury in chickens induced by bisphenol A and selenium deficiency-Mitochondrial reactive oxygen species and mitophagy-apoptosis crosstalk homeostasis. The Science of the total environment, 905: 166890. https://doi.org/10.1016/j.scitotenv.2023.166890

Ponamarev V., Popova O., Kostrova A., Agafonova L. 2023. The Concept of Development of New Ecologically Based Methods of Diagnostics and Pharmacocorrection in Veterinary Medicine (on the Example of Pathologies of the Hepatobiliary System). In *II International Conference "Sustainable Development: Agriculture, Veterinary Medicine and Ecology"* (VMAEE-II-2023). Karshi, 3011: 20028. https://doi.org/10.1063/5.0161092

Prusakov A., Zelenevskiy N., Shchipakin M., Bylinskaya D., Barteneva Y., Vasil'yev D., Stratonov A., Khvatov V. 2020. Morphology of the Vascular Bodies of the Encephalon's Ventricles of Cow (Bos Taurus Taurus). Macedonian Veterinary Review, 43(1): 31-36. https://doi.org/10.2478/macvetrev-2020-0011

Prusakov A.V., Zelenevsky N.V., Shchipakin M.V., Bylinskaya D.S., Vasiliev D.V. 2019. Comparative ultrastructural organization of the blood-brain barrier of birds and mammals. Morphology, 156 (6): 114.

Prusakova A.V., Zelenevskiy N.V., Prusakov A.V., Yashin A.V., Ponamarev V.S. 2024. Ultra-structural organization of the gallbladder mucous membrane of Anglo-Nubian goat. Veterinary research forum : an international quarterly journal, 15(3): 165-169. https://doi.org/10.30466/vrf.2024.2011081.4005

Prusakova A.V., Zelenevskiy N.V., Prusakov A.V., Yashin A.V., Ponamarev V.S. 2023b. Ultrastructural organization of liver hepatocytes of the Anglo-Nubian goat. Veterinarski Glasnik, 77(2): 176-187. https://doi.org/10.2298/VETGL230615007P

Reynolds E.S. 1963. The use of lead citrate at hight pH as an electronopaque stain in electron microscopy. The Journal of cell biology, 17(1): 208-212. https://doi.org/10.1083/jcb.17.1.208

Semchenko V.V., Samuseva R.P., Moiseeva M.V. 1999. International histological nomenclature. Omsk: Kolosova.

Strizhikov V.K., Strizhikova S.V., Sytko V.V. 2021. Comparative morphological characteristics of the hindbrain in domestic chickens and anseriformes. Current issues and ways to solving them in veterinary medicine and animal husbandry: Collection of materials from the International scientific and practical conference dedicated to the 90th anniversary of the birth of Professor Yu. F. Yudichev, Tyumen, Russia, 1, 89-96.

Yashin A.V., Prusakov A.V. 2021. Features of the state of the microcirculatory bed and membrane digestion in newborn calves with dyspepsia. International Bulletin of Veterinary Medicine, 2: 155-160. https://doi.org/10.17238/issn2072-2419.2021.2.155

Received: 08.09.2024.
Accepted: 16.04.2025.