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**REVIEW PAPER**

# THE IMPORTANCE OF TIGHT JUNCTIONS IN THE BLOOD-BRAIN BARRIER*

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**Abstract**

There are two barriers in the central nervous system (CNS) responsible for maintaining the homeostatic balance of the human body's internal environment in relation to external conditions: the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). The blood-brain barrier is a physical barrier with a fixed location between the blood and the nervous tissue. Its basic structural elements include endothelial cells of the brain capillaries, astrocytes and pericytes. The blood-brain barrier is the barrier between the blood and neurons which isolates the central nervous system from the rest of the body in a unique manner. The blood-brain barrier is formed by astrocytes and pericytes in addition to the endothelium. Endothelial cells are tightly connected to each other by tight junctions. These connections are composed of a protein complex, which includes claudins, occludins and tricellulines. The dominant protein in the blood-brain barrier is Claudin-5. Physiologically, this barrier is a transport centre for many molecules between the central nervous system and blood. Active transport

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takes place mainly with the help of specific transport mechanisms. Disorders of the blood-brain barrier have an impact on many diseases of the central nervous system, indicating negative effects of the destruction of the blood-brain barrier on the functioning of the brain. At the same time, the barrier is an obstacle for protein-derived chemotherapeutic agents, which prevents the effective treatment of many brain tumours. Currently, there is much research into methods of controlling the permeability of this barrier. The bibliometric on BBB and BCSFB the period 2011-2021 was utilized in the study. A literature review was carried out searching through the PubMed, SCOPUS and Google scholar. The criterion of thematic classification was implemented to carry out an analysis of bibliographic data.

**Keywords:** blood-brain barrier, central nervous system, active transport, intercellular connections, tight junctions, claudins.

## INTRODUCTION

The arrangement of cells in individual tissue systems is not accidental. It requires the creation of specific mechanisms that connect cells and facilitate the communication between them. Junctions are made of protein complexes built into the cell membrane. Depending on the location of the tissue in the body and the functions performed, their structure varies. There are several types of intercellular connections: barrier (closing, impenetrable), communicational and mechanical (desmosomes). The proper functioning of many organs depends on cell barriers formed by integral membrane protein complexes (Luissint et al. 2012, Villaseñor et al. 2019).

The blood-brain barrier (BBB) consists of capillaries, neurons, glial cells, and intercellular connections, including tight junction (TJ). The tight junctions create specific channels that selectively allow molecules and ions from the blood to pass to the neurons (Haseloff et al. 2015). They consist of claudins, occludins, tricellulins, and the family of zonula occludens proteins. The Claudin family consists of 27 different proteins (Suzuki et al. 2014). Their location and functions in different physiological processes vary significantly between the various molecules. All of them are both a structural and functional molecular component of TJ.

Thanks to tight junctions, BBB maintains a constant concentration of substances that are part of the cerebrospinal fluid, which ensures the proper functioning of the brain. The brain is an organ that is very sensitive to osmolarity fluctuations in the environment in which it is located, therefore even small changes cause impairment of its functioning. The term blood-brain barrier (German: Bluthirnschranke or Blut-Hirn-Schranke) was first introduced by the German neurologist Max Lewandowsky (Marcinowski 2020). This barrier is an important topic of interest in oncology and neurology as it prevents the passage of protein-derived drugs into neurons, resulting in poor results of chemotherapeutic agents in the treatment of brain tumours. Currently, researchers are mainly focused on finding methods to control the permeability of this barrier without destroying it: methods aimed

at obtaining increased BBB permeability, including therapies involving the use of multidrug resistance protein inhibitors.

## STRUCTURE OF THE BLOOD-BRAIN BARRIER

BBB is a physically dense structure made up of endothelial cells linked together by protein junctions called junctions. The endothelium is covered with pericytes (mesenchymal cells), capable of phagocytosis. The endothelium-pericytes complex is surrounded by a basement membrane, closely adjacent to the end plates of the astrocytes (glial tissue). The basement membrane consists of laminin, type IV and V collagen and fibronectin (Campisi et al. 2018).

The endothelium in the BBB forms a continuous layer and is a kind of specialized single-layer squamous epithelium. It provides a polarized lining on the inside of the capillary. The endothelium is rich in glycosaminoglycans, which are integrated with lipids and proteins of the cell membrane (enzymes, membrane receptors); it exhibits species, individual and organ specificity (Daneman 2012, Liu et al. 2012, Hajal et al. 2021).

The end caps of the astrocytes covering the BBB endothelium emit unidentified, as of yet, factors influencing the formation of tight junctions in endothelial cells. Moreover, they create additional protection that must be overcome by molecules passing through the BBB, thus increasing the impermeability of the barrier during increased neuronal activity (Serlin et al. 2015). This phenomenon is closely related to the depolarization of the astrocyte membrane. The outflow of $K^+$ ions from astrocytes increases the conductivity of $Ca2^+$ channels in the endothelium. The calcium ion current activates the segments of the cytoskeleton in endothelial cells, which in effect causes a contraction that widens the spaces between tight junctions (Brzezińska, Ziaja 2012).

An important element of the structure of the BBB are tight junctions, which constitute a complex of membrane proteins. They include claudins, occludins, tricellulines (Günzel, Fromm 2012, Luissint et al. 2012, Cummins 2020). Claudins forming the tight junction complex are responsible for specific transport in BBB (Daneman 2012); at least four different claudins are expressed in the endothelium: claudin-1; -3; -5 and -12 (Pfeiffer et al. 2011, Greene, Campbell 2016, Berndt et al. 2019, Greene et al. 2019). Apart from claudins, an important role is played by occludins, which, like claudins, bind to elements of the cytoskeleton thanks to cytoplasmic proteins of the zonula occludens (ZO-1, ZO-2, ZO-3) (Blasig, Haseloff 2011). ZO-1 is the first cytoplasmic protein closely related to BBB to have been discovered. Its molecular mass is 210-225 kDa, so it is much larger than occludin (65 kDa) and claudin (20-27 kDa). A few years after its discovery, two more isomers of the "closing

zone" proteins, named ZO-2 and ZO-3, were found. All proteins included in the ZO form the cytoplasmic complex of the so-called "scaffolding". C – the occludin end is connected to ZO-1, ZO-2 and ZO-3. These proteins are sequentially linked to cingulin (Luissint et al. 2012). The latest research on tight junctions is mainly based on the phase separation of zonula occludens from claudines. The results suggest that the transition of the "active phase" of ZO proteins into the condensed compartment associated with the cell membrane fuels the polymerization of claudins, resulting in a tight "ring" coalescence of tight junctions (Beutel et al. 2019).

The specific structure of the BBB protects the brain against the effects of neuroactive substances, e.g. catecholamines circulating in the circulatory system, and provides access to essential substances such as amino acids or glucose. The brain is ultimately supplied with nutrients straight from the blood thanks to selective transporters such as GLUT 1 located in the barrier (Patching 2017, Abdullahi et al. 2018).

The BBB is able to actively remove many lipophilic substances that could become a potential threat to the brain. The specific transport of substrates and metabolites takes place thanks to membrane transport systems. An example of a transport protein with broad substrate specificity is P-glycoprotein (P-gp). It is a 170 kDa transport protein belonging to the ABC family of transporters (ATP-binding cassette transporter), whose main function is to protect the body against xenobiotics by removing cytotoxic substances. Lipophilic toxins are immediately pumped from the brain environment into the bloodstream thanks to channels made of P-gp. BBB is also a structure that is difficult to break through in the immune system (Varatharaj, Galea 2017, Zhao et al. 2020). Its most important function is the precise rotation of chemical compounds between the central nervous system (CNS) and blood, so it protects the CNS against sudden spikes in the content and osmolarity of the cerebrospinal fluid during blood pressure fluctuations (Rosenberg 2012).

## TIGHT JUNCTIONS AS COMPONENTS OF THE BLOOD-BRAIN BARRIER

Specific TJs located in the BBB are morphologically different from those located in other epithelia. These differences are related to the greater number of mitochondria in BBB endothelial cells compared to other tissues and the extremely complex cross-linking of tight junctions. This discrepancy is related to the expression of specific claudins, therefore Claudin-3, -5, -1, -12 have been termed "blood-brain barrier proteins". At present, however, there is doubt about the presence of claudin-1 as the primary TJ-building protein in the BBB (Thomsen et al. 2017, Berndt et al. 2019).

Claudin-5 is an important component of TJ in BBB. The gene responsible for the expression of claudin-5 was originally named TMVCF. The name was associated with DiGeorge's disease, otherwise known as VCFS. In this anomaly, the Claudin-5 gene is not expressed, hence the original name of this protein (Li et al. 2021). During studies on Claudin-5-knock-out mice (i.e. mice lacking the gene responsible for the expression of claudin-5), it was observed that these animals are born alive, but die in the first 24 hours of life. The tests performed did not reveal any visible changes in the brain. Although the morphology of the TJ bands was correct, the BBB lost its "sealing" property for particles smaller than 800 Da (Paul et al. 2013, Greene et al. 2019). These studies proved that Claudin-5 is an essential barrier element as its presence affects the TJ sealing for molecules below 800 Da. The targeted manipulation of Claudin-5 should therefore not break the TJ structure but allow the BBB to be temporarily opened to small molecules (e.g. drugs) – Günzel, Yu 2013, Jia et al. (2014).

While the function of Claudin-12 in BBB is yet to be explained, the lack of Claudin-3 expression leads to significant morphological changes of TJ. These changes manifest themselves in a total breakdown of the diffusion barrier, and thus the breakdown of the TJ protein complex (Varatharaj, Galea 2017). Claudin-3 was known as RVP-1 (rat ventral prostate) because apart from BBB it is also found in the prostate, liver and enterocytes (Nakamura et al. 2019).

## PATHOLOGIES ASSOCIATED WITH DAMAGE TO THE BLOOD-BRAIN BARRIER

BBB is important for the proper functioning of the brain. Mechanical damage to it causes an increase in the concentration of calcium ions in the intercellular fluid, leading to a change in the location of proteins included in the TJ. The reorganization of the tight junctions in effect changes the morphology of endothelial cells. The proteins subject to most changes are occludin and zonula occludens (Coureuil et al. 2017). Disturbances in protein expression and endothelial reorganization lead to changes in BBB permeability. Hypoxia and ischaemia negatively affect TJ; phosphorylation occurs as a consequence of free radical activity. These processes lead to an increase in the distance between endothelial cells. Some post-translational modifications negatively affect the function of TJ, e.g. in occludin, phosphorylation at Ser / Thr residues does not alter the endothelial occludin location, while the identical post-translational modification at Tyr causes occludin degradation. The result is intra-plasmic disorders of connections with the proteins of the closing zone (ZO - 1, ZO - 2, ZO - 3), and thus the relaxation of the BBB (Bai et al. 2014). When the barrier loses its functions, the uncontrolled

transport of molecules to the brain takes place. The result is the pathophysiological sequelae of brain damage due to toxins and the change in the osmolarity of the cerebrospinal fluid. The main factors are: high concentration of bilirubin and glutamate in the blood, free radicals, glucose and oxygen deficiency and unregulated ion flow (Zhao et al. 2020).

BBB is immature at birth. This can be inferred from experiments on test animals, in which low-mass molecules penetrated the barrier more easily during neonatal development than in adulthood. Critical periods in BBB formation are difficult to distinguish, but it is generally believed that its formation begins after the formation of blood vessels in the nervous system. It should be noted that not all capillaries become impermeable in the same period of time. It mainly depends on the anatomical location – the barrier within the spinal cord is formed earlier than in the forebrain (Brito et al. 2014). An example of the gradual and regional "sealing" of the BBB is the jaundice of the subcortical nuclei, the so-called kernicterus. This disorder is more common in premature babies than full-term infants (Brito et al. 2014). In newborns with severe jaundice, in whom the concentration of free bilirubin in the blood is too high, and the BBB is not fully developed, the bilirubin penetrates the brain tissues, which results in irreversible damage to neurons and the glial tissue, especially astrocytes. An additional unfavourable factor in newborns is the immature system of liver enzymes responsible for binding bilirubin. The equivalent of this condition in the elderly is the Crigler-Najjar syndrome (congenital deficiency of glucuronide transferase). Bilirubin, free once albumin is cleaved off in the liver, is esterified by glucuronic acid with the involvement of glucuronic transferase to its soluble form called conjugated bilirubin. In people with the Crigler-Najjar syndrome, this process is disturbed, which can result in encephalopathy in extreme cases. The appearance of this disease in newborns indicates that BBB maturation is long-lasting and takes place also after birth (Goodall et al. 2018).

Recent studies indicate a relationship between cognitive impairment in the elderly and the state of BBB microcirculation in the brain. When microcirculation does not function properly as a result of processes such as ischaemia, hypoxia, oxidative stress, it causes further damage to the vascular endothelium and nerve cells (Costea et al. 2019, Stamatovic et al. 2019). BBB plays a key role in the immunopathogenesis of autoimmune neurological disorders as it is one of the few pathways of "entry" into the central nervous system (Hajal et al. 2021). BBB lesions contribute to pathology and may play an important role in the development of the most common neurodegenerative disease affecting the elderly, namely Alzheimer's disease (AD) – Erickson, Banks (2013). B-amyloid peptide, a major component of senile plaques in AD, influences the expression and localization of TJ proteins. Extracellular matrix and TJ are substrates of matrix metalloproteinases (MMPs), the activity of which is also increased in AD and during hypoxia, which results in a decreased expression of TJ proteins (Wan et al. 2014).

BBB damage is also involved in the progression of the second most common neurodegenerative disease, Parkinson's disease (PD). The influence of BBB dysfunction in PD is not widely studied, despite the influence of known mechanisms and factors damaging BBB, such as oxidative stress and MMP, in the pathomechanism of the disease (Pan, Nicolazzo 2018).

In the current pandemic situation, the latest research on breaking the BBB by the S protein of the SARS-CoV-2 coronavirus spike (Buzhdygan et al. 2020) is important. Modelling studies have shown that the viral S1 protein promotes the loss of BBB integrity, and subsequent analysis has shown that SARS-CoV-2 peak proteins have the ability to induce a pro-inflammatory response in brain endothelial cells that may contribute to an altered state of BBB function. These results could therefore help explain the neurological consequences seen in COVID-19 patients.

## THERAPIES MODULATING THE PERMEABILITY OF THE BLOOD-BRAIN BARRIER

The biochemistry of the environment surrounding neurons in the CNS is carefully regulated. Substances dissolved in plasma, e.g. low molecular weight gases: ($O_2$, $CO_2$), lipophilic compounds (e.g. ethanol), steroid and thyroid hormones, opioid analgesics and peptides weighing 400 - 800 Da, reach the internal environment of the brain through simple diffusion (Liu et al. 2012). The remaining substances are selectively transported in a controlled manner, thanks to the activity of the BBB barrier. The rate of penetration of compounds into the brain tissue is inversely proportional to the size of the particles and directly proportional to their affinity for fat (Campos-Bedolla et al. 2014): low-molecular, lipophilic compounds are absorbed at a high rate, and high-molecular, polarized, hydrophilic compounds cross the barrier much slower.

As mentioned earlier, P-gp has the task of transporting xenobiotics out of the brain environment; however it also removes chemotherapeutic agents. Many cancer cells also have these channels, which pump out chemotherapeutic agents administered during therapy out of the brain. In the instance of a brain tumour, this is an extremely unfavourable phenomenon, referred to as multi-drug resistance (Zihni et al. 2016). P-glycoprotein is also called a multidrug resistance protein 1 (MDR1) due to the drug resistance of the cancer cell lines in which it is overexpressed. It is a common feature of many multi-drug resistant tumours (Calatozzolo et al. 2012), demonstrated, among others, in glioma, and levels of overexpression were correlated with multi-drug resistance and tumour staging (De Trizio et al. 2020).

Currently, many studies are devoted to therapies aimed at regulating BBB permeability (Azad et al. 2015). Increasing the permeability of BBB can

be indicated in various ways: the change in osmolarity triggers a therapeutic window of several hours, administration of bradykinin increases the permeability of endothelial cells. Increased migration of chemotherapeutic agents through the BBB can be achieved thanks to positive pressure microperfusion, the use of drug carriers in the form of viral vectors, liposomes, or other carrier molecules, or with the use of physical methods: ultrasounds under MRI guidance to disrupt proteins in tight junctions or radiation for the purpose of inducing focal lesions and increasing BBB permeability within the tumour. The most promising seems to be the regulation of the activity of specific surface proteins on the capillary endothelium of cells, in particular the modulation of the P-gp protein. The use of inhibitors or substances modulating multidrug resistance proteins may offer more targeted strategies and drug delivery systems to the CNS less burdensome for the patient. One of the promising substances that binds the P-gp protein in a non-competitive way, even at nanomolar concentrations, is tariquidar (Weidner et al. 2016). Intensive research has been carried out on this substance for years in order to accurately determine its characteristics as a drug.

## CONCLUSIONS

BBB is one of the most impermeable barriers in the human body. This is largely due to its specific structure. Particular attention should be paid to the intercellular connections such as TJ. Tight junctions consist of narrow bands of membrane proteins, e.g., claudins. They create a very tight barrier preventing the free diffusion of molecules. The BBB is not just an impermeable partition, but an active transport centre between the CNS and the circulatory system for many molecules. In the light of the cited studies, BBB dysfunctions induce many diseases of the central nervous system. It should be emphasized, however, that the mechanism of opening the barrier, without its complete destruction, has not been fully explained yet. This is of great importance for patients with an inoperable brain tumour, or ones post tumour removal with a residual tumour left behind, when they are administered a chemotherapeutic agent, where it is important that the active substance is able to break through the BBB and reach the target tissues. In order to understand the entire process, it is necessary to continue research in this area.

**Conflict of interest**

The authors declare no potential conflict of interest concerning the authorship and/or publication of this article.

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