Chapter

Blood-Brain Barrier Dysfunction in the Detrimental Brain Function

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Abstract

The blood circulation interface and the neural tissue feature unique characteristics encompassed by the term blood -brain barrier (BBB). The barrier's primary functions are maintenance of brain homeostasis, selective transport, and protection, all of them determined by its specialized multicellular structure. The BBB primarily exists at the level of the brain microvascular endothelium; however, endothelial cells are not intrinsically capable of forming a barrier. Indeed, the development of barrier characteristics in cerebral endothelial cells requires coordinated cell–cell interactions and signaling from glial cells (i.e., astrocytes, microglia), pericytes, neurons, and extracellular matrix. Such an intricate relationship implies the existence of a neurovascular unit (NVU). The NVU concept emphasizes that the dynamic BBB response to stressors requires coordinated interactions between various central nervous system (CNS) cell types and structures. Every cell type makes an indispensable contribution to the BBBs integrity, and any cell's failure or dysfunction might result in the barrier breakdown, with dramatic consequences, such as neuroinflammation and neurodegeneration. This chapter will focus on the structure and function of the BBB and discuss how BBB breakdown causes detrimental brain function.

Keywords: neurovascular unit, neurovascular coupling, BBB breakdown

1. Introduction

The interface between the blood circulation and the central nervous system (CNS) comprises complex multicellular structures with unique features that selectively allow or restrict the passage of substances between these compartments. Two distinct blood CNS barriers exist: the endothelial blood-brain barrier (BBB), localized at all levels of the cerebral vascular tree, and the epithelial blood-cerebrospinal fluid barrier, situated at the choroid plexuses within the brain's ventricular system, separating the brain interstitial fluid (ISF) and the cerebrospinal fluid (CSF) from the peripheral circulation [1].

The BBB is a term used to describe the unique properties of the microvasculature of CNS. The vascular tree are continuous non fenestrated vessels and contain a series of properties that allow them to tightly regulate the movement of molecules, ions, and cells between the blood and the CNS [2, 3]. The human brain is one of

the most metabolically active organs in the body, under physiological conditions, the human brain receives 20% of the total basal cardiac output and uses 20% of the body's oxygen and glucose [4]. Energy substrates are consumed by the brain from the blood via transport across the BBB, as the brain lacks a metabolic reservoir to store macromolecules for use when needed. In the mammalian brain, cerebral arteries, arterioles, and capillaries supply CNS with blood in response to neuronal stimuli by increasing the rate of cerebral blood flow (CBF), nutrients and oxygen delivery, a mechanism known as neurovascular coupling [5].

The neurovascular coupling requires an integrated multicellular response to provide the perfusion needs for neuronal metabolism [5], different cell types are involved in this action, neurons and astrocytes generate mediators that trigger cellular responses in endothelium cells, pericytes, and smooth muscle cells (SMC), which contribute to vascular response in the BBB permeability. Functionally, these interactions are included in the concept of the neurovascular unit (NVU), which comprises various central and peripheral cell types that contribute to BBB structure and function (**Figure 1**) [6, 7]. However, in pathophysiological states, BBB breakdown and dysfunction leads to leakages of harmful blood components into the cerebral parenchyma, cellular infiltration, and aberrant transport and clearance of molecules [8], which is associated with CBF reductions and dysregulation [9], contributing to neurological effects.

Here, we first examine the cellular components that underlie the establishment of the BBB in NVU. Then, we focus on the cellular components of BBB and transport physiology. Complementary and in a translational way, examine how BBB breakdown and dysfunction related to acute vascular CNS disorders such as ischemic and hemorrhagic stroke, and BBB breakdown and dysfunction relate to neurological deficits and other pathologies in Alzheimer's disease (AD), Parkinson's disease (PD) and multiple sclerosis (MS).

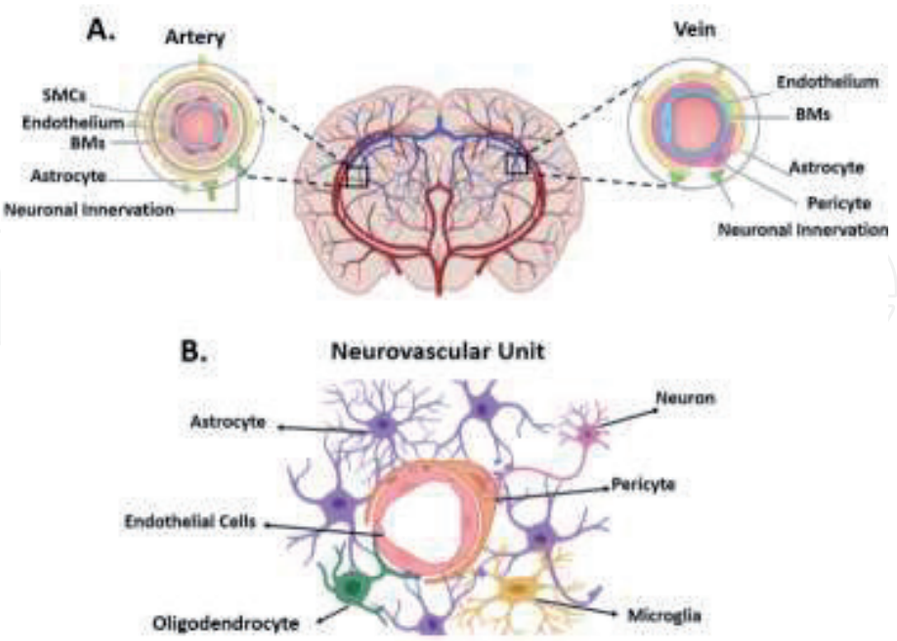

Figure 1.
(A) The multicellular structure of the neurovascular unit (NVU). The BBB is formed by endothelial cells at the level of the cerebral bed (arterial and venous). These endothelial cells interact with perivascular elements, such as the basal lamina (BMs), smooth muscle cells (SMCs) and astrocytic end-feet processes, perivascular neurons and pericytes to form a functional BBB. (B) The core anatomic elements of the NVU. Created with BioRender.com.

2. The BBB and the neurovascular unit

The NVU is a relatively recent neuroscience concept, representing the structural and functional multicellular relationship between the brain and blood vessels [5]. The cellular components are the endothelial cells (EC), pericytes, perivascular astrocytes, microglia, the basement membrane (BM), and neuron (**Figure 1**) [10]. The NVU components share intimate and complex associations, and these associations have led to their classification as a single functioning unit. The NVU is responsible for the maintenance of a highly selective BBB and cerebral homeostasis, as well as the control of CBF [11]. Each NVU component seems to play a specific and active role, maintaining the dynamic linkages reciprocally under physiological conditions.

Endothelial cells are considered the BBB's anatomic basis since they form and tightly seal the wall of all cerebral vessels, thereby building a physical barrier between the blood and the brain parenchyma (**Figure 1**). Two different types of endothelial junctions exist: adherens junctions (AJ) and tight junctions (TJ) [12]. Adherens junctions comprise vascular endothelial (VE) cadherin and neural (N-) cadherin, both acting via homophilic interactions [13]. While VE-cadherin is vital for sealing adjacent endothelial cells, N-cadherin mediates their association with pericytes [13]. TJ contains transmembrane proteins such as claudins, occludins, and junction adhesion molecules, as well as the zona occludens cytoplasmic proteins (ZO). These proteins act collectively to close off interconnecting endothelial cells [14], restricting the paracellular diffusion of hydrophilic substances, even ions; this is a unique feature of the BBB endothelium [11] in the other hand, the neurovascular endothelial cells, in contrast to peripheral endothelial cells, is the low expression of adhesion molecules (e.g. member of the immunoglobulin superfamily VCAM-1), in this sense, immune cells never cross unstimulated BBB in the healthy CNS [15]. Interactions of endothelial cells with other NVU members mediate a decrease in transcytotic activity, downregulation of leukocyte adhesion molecules, and regulation of interendothelial junction stability during development and adulthood [14].

Pericytes are mural cells enwrapping capillary blood vessels on their abluminal side. Structurally, pericytes extend processes from their cell body, covering several endothelial cells (**Figure 1**). In contrast to peripheral tissues, the brain has the highest pericyte to endothelial cell ratio [16]. Pericytes are embedded within the basement membrane (BM) of capillary endothelial cells and are thus centrally positioned between endothelial cells, astrocytes, and neurons [3]. In total, pericytes cover a large cerebral vascular area which can reach up to 40% of the neurovascular surface [17]. One of the main functions of pericytes is the control of the vasoreactivity and cerebral blood flow in response to neuronal activity [18]. As a recently explored example, glutamate induces prostaglandin E2 and nitric oxide release, which leads to actively relaxing pericytes to dilate capillaries [19]. Vascular permeability increases with decreasing pericyte coverage, which is partly due to the regulation of endothelial transcytosis. Moreover, other parts of the NVU are also influenced by pericytes, including neurons, immune cells, and the basement membrane [20].

Astrocytes are the most abundant cell type in the brain with a variety of functions. Beyond BBB regulation, they participate in synapse formation, uptake and recycling of neurotransmitters and ions, regulation of extracellular potassium levels, nutrition of neurons, and control of inflammatory responses within the CNS [21]. Astrocytes provide a cellular link between the neuronal circuitry and blood vessels. This neurovascular coupling enables astrocytes to relay signals that regulate blood flow in response to neuronal activity; this includes regulating the contraction/dilation of vascular SMC surrounding arterioles and

capillaries [22, 23]. Astrocytes are also critical cellular support of BBB integrity. Recent molecular studies have shown several molecules released by astrocytes that enhance and maintain barrier tightness, such as cholesterol and phospholipid transporter molecule apolipoprotein E [24, 25]. Release of apolipoprotein E from astrocytes, for example, regulates endothelial TJs by signaling through the low-density lipoprotein receptor related protein 1 (LRP1) on both pericytes and endothelial cells of CNS microvessels [25]. Astrocytes have been identified as essential mediators of BBB formation and function because of purified astrocytes' ability to induce barrier properties in non-CNS blood vessels [26]. Based on these observations, it has been proposed that astrocytes are necessary for the formation of impermeable TJs in the developing vessels of the BBB.

Microglia derive from hematopoietic precursors that migrate from the yolk sac into the CNS parenchyma, acting as the brain's main line of defense past the BBB and play a vital role in innate immune responses in the vascular bed and cerebral parenchyma (**Figure 1**) [27], little is known about how microglial-endothelial communications may shape and regulate the homeostatic BBB. However, studies have demonstrated that microglia are associated with endothelial's nascent vessels in the developing brain, and promote the fusion of cells in the stages following vascular endothelial growth factor-mediated induction [28]. Recent studies have shown the activation of microglia in CNS disorders like AD and multiple sclerosis, which are associated with BBB breakdown and neuroinflammation. In these conditions, microglial activation may be both a cause and consequence of BBB dysfunction [20]. Microglia can exist in one of two active states: in the activated pathway, microglia release proinflammatory cytokines like interleukin-1b and tumor necrosis factor-a. Whereas in alternative pathways, microglia are involved in tissue repair, phagocytosing neurons and foreign material, releasing chemokines and vascular endothelial growth factor [29]. On the other hand, brain endothelial cells can also secrete molecules that cause microglial activation [30]. In summary, a complex interplay between systemic and CNS derived immune cells exists at the BBB.

Basement Membrane: The vascular tube is surrounded by two basement membranes (BMs), the inner vascular BM, and the outer parenchymal BM (**Figure 1**). The vascular BM is an extracellular matrix secreted by the ECs and pericytes, whereas the parenchymal BM is primarily secreted by astrocytic processes that extend towards the vasculature [31]. These BMs consist of different molecules, including type IV collagen, laminin, heparin sulfate proteoglycans, and other glycoproteins [32]. They provide an anchor for many signaling processes in the vasculature and also constitute an additional barrier for molecules and cells to cross before accessing the neural tissue. Disruption of these BMs by matrix metalloproteinases is an integral part of BBB dysfunction and posterior leukocyte infiltration, which can be observed in many different neurological disorders [32].

Neurons and interneurons. Neurons can detect small variations in their supply of nutrients and oxygen and transform these signals into electrical and chemical messages to adjacent interneurons or astrocytes. In response to these signals, necessary adjustment mechanisms are initiated. Due to this phenomenon, neurons are considered NVU's pacemaker [15]. Neurons need to be able to signal to cerebral vessels when their energy demands change. Positive and negative feedback mechanisms exist to regulate cerebral blood flow, accompanied by adjustments of substrate delivery across the BBB, a process known as neurovascular coupling [33]. In this sense, one relevant mechanism for neurovascular coupling is direct innervation of astrocytic processes or the endothelial tube by, amongst others, serotonergic, noradrenergic, cholinergic, and GABAergic neurons [4]. Mechanisms of neurovascular coupling, particularly those that can explain direct molecular effects on BBB integrity, are yet to be established. Future knowledge will be of great interest since

new therapeutic tools could help modulate intercellular communication in diseases linked to vascular dysfunction.

3. BBB physiology: building blocks and transport routes in BBB

3.1 BBB junctional molecules

The BBB is a diffusion barrier essential for the normal function of the CNS. The NVU endothelial cells differ from endothelial cells in the rest of the vascular system by their absence of fenestrations, and for having more extensive junctional molecules, mainly TJ, and sparse pinocytic vesicular transport [34]. These junctional molecules limit the paracellular flux of hydrophilic molecules across the BBB. In contrast, small lipophilic substances (O2 or CO2) can diffuse freely across plasma membranes along their concentration gradient [34]. Nutrients such as glucose and amino acids enter the brain via transporters, whereas receptor-mediated endocytosis mediates larger molecules' uptake, including insulin, leptin, and iron transferrin [35], it is believed that all the components of the BBB are essential for the normal function, stability, and permeability of the BBB.

The Junction complex in the BBB comprises TJ, AJ, and Gap junctions (GJ). The TJ ultrastructurally appear as apparent fusion sites, involving the outer plasma membrane of adjacent endothelial cells [36]. The number of TJ strands, as well as the frequency of their ramifications, varies and consists of three integral membrane proteins: claudin, occludin, and junction adhesion molecules, as well as several other cytoplasmic accessory proteins, including members of the family zonula occludens (ZO-1, ZO-2, ZO-3) and cingulin (**Figure 2**). Cytoplasmic proteins link membrane proteins to actin, for maintaining the structural and functional integrity of the endothelium [36]. The Claudins were identified as the principal component of TJ and are localized exclusively at TJ strands. Claudins bind to other claudins on adjacent endothelial cells to form the primary seal of the TJ [37]. Closest to the

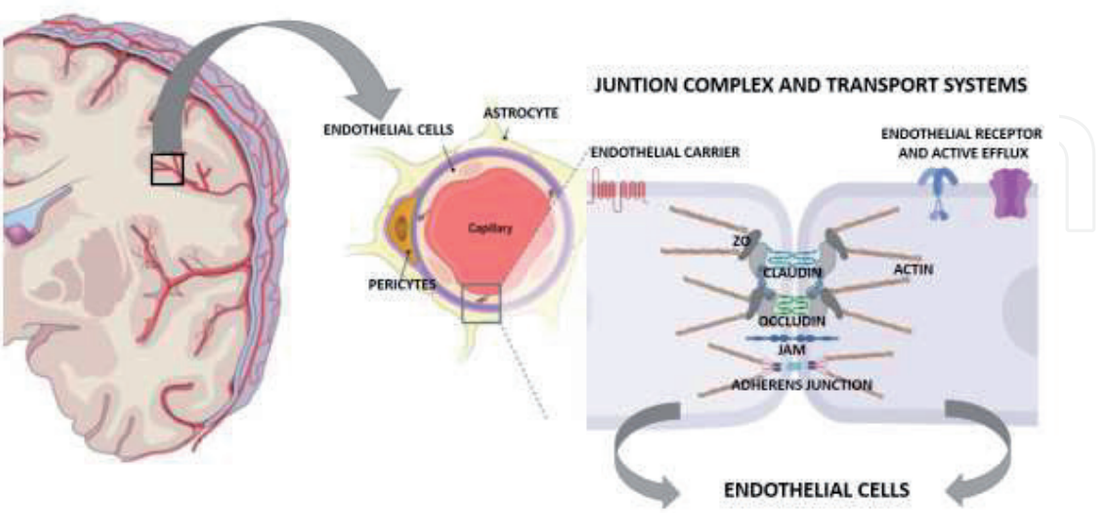

Figure 2.
Basic molecular organization of BBB junctional molecules and transport. The endothelial cells confer unique properties on the BBB. They are the principal line of cerebral vasculature and have numerous junctional molecules such as tight junctions, adherens junction, gap junctions and accessory proteins that limit the passive paracellular diffusion of all but the smallest of solutes and ions. On the other hand, carriers, receptors and active efflux protein mediated transport allow substances such as peptides, amino acids, and glucose to selectively cross the BBB and release toxic substances and drugs into the blood preventing them from entering the brain. Created with BioRender.com.

apical membrane, the claudin 1, 3, 5, 12, and occludins limit paracellular diffusion of solutes and ions across the BBB [38]. Loss of claudins is associated with permeability and BBB breakdown in neurodegenerative disorders and acute CNS diseases [39]. TJ proteins connect to actin and vinculin-based cytoskeletal filaments via scaffolding proteins of the membrane, associated with ZO 1, 2, and 3 [40]. Previous studies have shown that ZO-1 deficiency leads to BBB breakdown in many neurodegenerative and acute CNS disorders [41]. Occludin, another integral protein localized at the TJ, form the TJ's paracellular barrier when conjoined with neighboring cells' claudins [42]; the cytoplasmic domain of occludin directly associates with ZO proteins. The expression of occludin has also been documented in human adult brains, but not in average human newborn and fetal brain, suggesting their role as regulatory proteins that can alter paracellular permeability of the BBB [35]. The third type of TJ-associated membrane protein, junctional adhesion molecules (JAM), structurally consists of a single transmembrane domain and an extracellular portion with two immunoglobulin-like loops joined by disulfide bonds [43]. Three JAM-related proteins, JAM-1, JAM-2, and JAM-3 are expressed in human BBB and previous studies have shown their participation in cell-to-cell adhesion and monocyte transmigration through BBB [44].

The AJs are established between neighboring cells by homophilic interactions between the transmembrane proteins, vascular endothelial cadherin (VE-cadherin), and epithelial cadherin (E-cadherin) in CNS [13]. Nearby to the basolateral membrane, AJ proteins, VE-cadherin, and platelet endothelial cell adhesion molecule (PECAM-1) form homophilic endothelial-to-endothelial contacts limit paracellular diffusion of solutes [13]. GJ are other junctional molecules, whose connexin-37 (CX37), CX40, and CX43 form hemichannels between endothelial cells [45]. These membrane proteins enable direct cytoplasmic exchange of ions and low molecular weight metabolites between adjacent cells; these channels of communications are essential for propagating electrical signals and coordination of cell signaling by transfer of second messengers [46]. Furthermore, brain endothelial GP also support tight junction integrity.

3.2 BBB transport systems

The major BBB transporters, receptors, and channels found in endothelial cells and pericytes have been validated by transcriptomic studies and protein analysis (**Figure 2**) [34]. Except for gases and small lipophilic molecules that freely diffuse across the endothelium, brain endothelial transport systems regulate molecular exchanges between blood and brain. The BBB's highly selective nature and the high metabolic demand of the brain demand other routes of entry for various nutrients to feed and nurture the brain [34]. Metabolic supply is achieved via several transporters expressed on the surface of CNS endothelial cells that drive the active transport of specific solutes and metabolites into the brain [47]. On the other hand, given the close proximity and highly interactive signaling between vascular pericytes and endothelial cells, it is relevant to describe in this chapter the BBB pericyte transporter.

Endothelial carrier enables solutes such as carbohydrates, amino acids (AA), monocarboxylic acids, hormones, fatty acids, nucleotides, inorganic ions, amines, choline, and vitamins to cross the BBB via substrate-specific transporters (**Figure 2**). In terms of carbohydrate transporters, GLUT1 (glucose transporter 1) is a uniporter that transports glucose. GLUT1 can transport glucose (and other hexoses) from either side of the luminal and abluminal endothelial membrane extracellularly or intracellularly [48]. Since glucose is lower in the brain interstitial fluid (ISF) than plasma, GLUT1 favors blood-to-brain transport of circulating glucose. GLUT1 is

expressed in endothelial cells, but not in neurons. Their importance is best illustrated by the fact that transcript encoding GLUT1 is one of the most abundant transcripts in brain endothelium. Their dysfunction and lack cause barrier breakdown and can prevent clearance of amyloid plaques, suggesting a contributing role in Alzheimer's disease progression [49].

Regarding the transport of amino acids, all essential AA are transported into the brain across the BBB via endothelial AA transporter 1 and 2 (LAT1/2), that transport bidirectionally neutral AA such as tryptophan and tyrosine [50], and the cationic AA transporter 1 and 3 (CAT1/3) that transport cationic AA such as lysine and arginine [51]. Also, on the abluminal membrane transporters for excitatory AA (EAAT1/2/3) transport glutamate and aspartate out of the brain, limiting their excitotoxic effects on neurons [52]. Transporters of neutral and excitatory AA, such as glycine, taurine, and GABA are enriched abluminally and with high-affinity transport from brain to endothelium in a sodium dependent manner, and then, these AA are transported across the luminal membrane of the BBB into the blood via low-affinity transporters into the circulation [53]. Finally, essential fatty acids are essential for brain development and postnatal neural functions. The Brain endothelium expresses luminal transporters for fatty acids, including fatty acid transport protein 1 and 4 (FATP- 1/4) and the MFSD2A (Major Facilitator Superfamily Domain containing 2a) [54]. In the brain, MFSD2a is exclusively expressed in brain endothelium and is required for right BBB development and functional integrity. Finally, for Lactate released from skeletal muscles during exercise, and ketone bodies derived from liver from metabolism of fatty acids, the transport is facilitated by monocarboxylate transporter-1 (MCT1). Once inside the brain parenchyma, they are used as alternative energy metabolites by the brain, supply the brain with key substrates for DNA and RNA synthesis [54]. Nucleotides and nitrogenous base, e.g., cytosine, guanine, adenine, thymine and uracil, are all transported across the BBB via sodium-independent concentrative nucleoside transporter-2 (CNT2) and the sodium-independent equilibrative nucleoside transporter-1 and 2 (ENT1/2) [55].

Endothelial receptor is the most important transporter because proteins and large macromolecules (e.g., fibrinogen, immunoglobulins, thrombin, plasminogen, and growth factors) cannot cross the BBB. However, some proteins and peptides use receptor transport to cross the BBB and enter the brain (**Figure 2**). Transferrin receptor (TfR) [56], insulin receptor (IR) [57], and leptin receptor (LEP-R) [58] mediate blood-to-brain transport of transferrin (iron-protein carrier), insulin, and leptin across the BBB, respectively. This characteristic has promoted its use for CNS drug delivery, including therapeutic antibodies [59]. Receptors LRP1 and LRP2 are expressed in the BBB's brain endothelium, with LRP1 binding Alzheimer's soluble Ab fragments and mediating its brain-to-blood clearance [60].

Endothelial active efflux and ion transport. ATP-binding cassette (ABC) transporters utilize ATP as an energy source and are expressed at the luminal side of the BBB endothelium. They function to prevent brain accumulation of drugs, xenobiotics and macromolecules via active efflux from endothelium to blood [**Figure 2**]. Some examples are ABCB1 (also known as P-glycoprotein, P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-associated proteins (MRP). The BBB also has a significant role in regulating ions' concentration in the CNS. The luminal sodium pump, Na^+K^+ATPase, is a key regulator of sodium influx into the brain and potassium efflux from the brain, maintaining high concentrations of Na^+ and low concentrations of K^+ in the brain, critical for the electrophysiological equilibrium of the resting membrane and action potentials [61].

Luminal Na-K- Cl (chloride) cotransporter (NKCC) mediates entry of Na^+, K^+ and $2Cl^-$ from blood-to-endothelium. The bicarbonate $(HCO_3)^-$Cl exchanger

mediates the entry of intracellular Cl^- and the extracellular release of HCO_3^-, regulating intracellular endothelial pH levels [62]. The Na^+-Ca^{2+} (sodium-calcium) exchanger cotransporter mediates Ca^{2+} efflux from endothelium into brain ISF, which maintains low intracellular Ca^{2+} levels in the microvascular endothelium [34]. Abluminal transient receptor potential (TRP) channels, also known as non- selective Ca^{2+} conducting cation channels, are expressed in both arterial endothelium and brain microvascular endothelial cell lines. TRP channels regulate Ca2 influx into brain endothelium, which in turn promotes the release of soluble factors such as NO, prostaglandins, and endothelial-derived hyperpolarizing factor initiating endothelium-dependent vasodilation [63]. BBB dysfunction also generates a leak of molecules across it, enabling considerable vascular fluid movement across the microvascular endothelium and the development of vasogenic edema [64]. Increased expression and activity of Na-K-Cl cotransporter (NKCC), sodium-hydrogen antiporter 1 and 2 (NHE1 and NHE2), and TRP channels promote the influx of Na^+, and Cl^-, generating a subsequent gradient osmotical that force the water movement across the BBB.

Pericyte transporters. Recent studies suggest that pericytes also express several transporters, receptors, and ion channels (**Figure 2**), such as carbohydrate transporters like the insulin-regulated glucose transporter GLUT4 and GLUT10 [65] and AA transporters, including the high-affinity excitatory AA transporter EAAT2, sodium-dependent neutral AA transporter SLC6A17, sodium and chloride dependent transporter SLC6A20 for small AA including glycine and proline, GABA transporter-1 and 2 (GAT1; GAT2), and the cationic AA transporter CAT2 [34, 65]. These transporters contribute to the removal of excitatory AA from the brain to prevent excitotoxicity, similar to endothelial transporters.

As endothelial cells, pericytes express lipoprotein receptor LRP1, mediating cellular uptake followed by its intracellular degradation and clearance [66] Pericytes regulate cerebrovascular integrity in an APOE-dependent way, inhibiting the proinflammatory CypA-MMP-9 pathway which prevents the degradation of BBB's TJ and basement membrane proteins [67]. These findings support that pericytes play an active role in regulating CBF and permeability of the BBB.

4. BBB dysfunction

BBB's integrity is essential for the normal functioning of the nervous system. It comes as no surprise then that its disruption initiates and perpetuates several neurological pathophysiological events. Although the nature and extent of such changes vary from every condition, one key commonality is the breakdown of BBB and the detrimental functioning of the NVU [4]. The BBB prevents neurotoxic plasma components, blood cells, and pathogens from entering the brain (integrity of BBB). At the same time, the BBB regulates transport of molecules into and out of the central nervous system (CNS) (permeability of BBB). In cerebrovascular diseases, BBB breakdown and dysfunction leads to leakages of components into the CNS, contributing to neurological deficits [68].

The cells of the NVU are extremely sensitive to a number of different substances, including pro-inflammatory cytokines (e.g. IL-1, IL-6, TNF-α, interferon-γ), lipid mediators, oxidative compounds (free radical), vasogenic agents (e.g. glutamate, serotonin, histamine) and other endogenous stimuli (e.g. extracellular K^+ and intracellular Ca^{2+}). Many of these substances are released under pathophysiological conditions and changes of their levels in BBB is a critical event in the development and progression of CNS dysfunction [69]. In some cases, increased BBB permeability is a consequence of the pathology, such as with ischemic stroke

and traumatic brain injury, increased of intrinsic cellular proinflammatory, oxidative stress and dysregulation of vasogenic mediators, whereas in other cases BBB opening may be another condition in which cerebrovascular abnormalities have been noted, such as neurodegenerative disease [70]. As a result, there is a direct association between integrity impairment and high permeability of these substances in the brain. Some of the steps that follow include alteration or breakdown of the physical, transport, and immune barriers.

4.1 Alteration of BBB by cerebrovascular injury

4.1.1 Ischemic stroke

In ischemic stroke, there is a sudden cessation of blood supply to the brain tissue, which translates into reduced oxygen and glucose delivery, both essential for ATP production. Depletion of ATP levels can lead to impaired functioning of Na/K-ATPase and Ca^{+2}ATPase activity, generating ion- gradient failure and abnormal intracellular ion accumulation. By contrast, endothelial transporters' activity, such as Na/H ion-exchanger and Na-K-Cl cotransporter are stimulated. This secondarily induces increased Na^{+}, Cl^{-}, and water across to the barrier and into the brain parenchyma, which results in characteristic cytotoxic edema secondary to ischemia [71]. The stimulation of this transporter's activity also triggers endothelial cell Na^{+} accumulation, generating swelling that contributes to BBB breakdown [72]. The Na^{+} cellular uptake depolarizes the cell's membrane, opening voltage-gated ion channels and promoting Ca^{2+} further cell uptake. These changes, in turn, prompt the release of excitatory neurotransmitters, which can be toxic [71]. BBB's breakdown in stroke occurs in a biphasic subacute fashion [70]. In the initial hit, activated metalloproteinases MMP-2 attack tight junction proteins. This activation is mediated by membrane-type MMP (MMP-14) and the fur gene expression, regulated by hypoxia-induced factor 1a (HIF-1a) [73]. Decreased expression and disorganization of tight junction constituent proteins, claudins, are the first signs of BBB damage, with further dysfunction of influx and efflux BBB transporters' expression. These changes are limit the hypoxic area and revert after the acute insult [70].

After 24 and 48 hours post-reperfusion, a non-reversible second phase takes place. Proinflammatory local cytokines activate inducible and freely available metalloproteinases MMP-3 and MMP-9, whose destructive activity characterizes this phase [74]. The most abundant cytokines present in focal cerebral ischemic areas are TNF-alfa and IL-1b [75] and have also been observed to decrease the expression of occludin and ZO-1 [76]. Cyclooxygenase-2 also plays a role in this second and more harmful opening of the BBB. Although this inflammation is local and mainly initiated by the activation of glia and pericytes, the BBB's damage and opening allow monocytes and neutrophils' entrance, perpetuating and amplifying the local inflammatory response [74]. The breakage of BBB in ischemic stroke is also the precursor of further complications such as the hemorrhagic transformation of the infarcted parenchyma [77]. A schematic view of ischemic stroke and intracerebral hemorrhage mechanisms are shown in **Figure 3**.

BBB can also be disrupted by the action of reactive oxygen species (ROS) and ensuing oxidative stress. Superoxide anion (O^{2-}) is a known mediator of cellular damage after ischemic stroke. Under oxidative stress conditions such as stroke, superoxide dismutase's (SOD) metabolic capacity of controlling the biological activity of O^{2-} gets surpassed. When combined with nitric oxide (NO), O^{2-} forms peroxynitrite, a cytotoxic and proinflammatory molecule that can initiate and amplify BBB's injury by its ability to nitrosylate tyrosine and inducing endothelial damage [78]. Oxidative stress plays a critical role in ischemia/reperfusion (I/R)

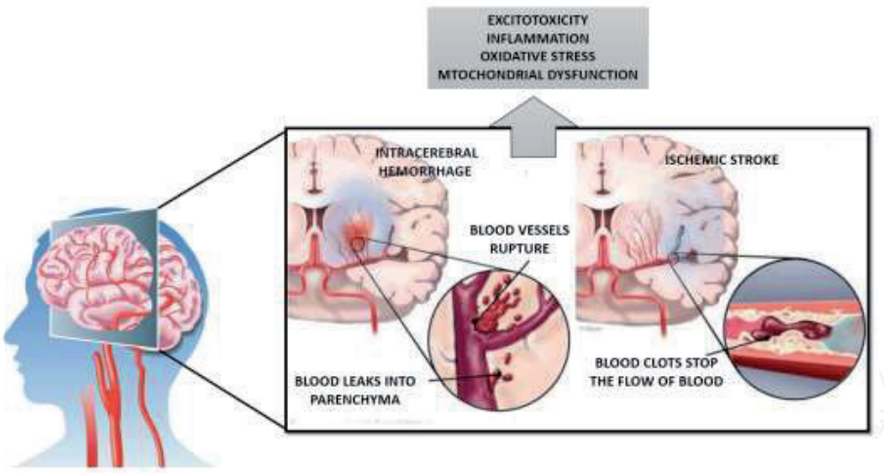

Figure 3.
A schematic view of ischemic stroke and intracerebral hemorrhage mechanisms. Activation of glutamate receptors following ischemic stroke leads to excitotoxicity and calcium influx, this impairs the neuronal homeostasis leading to activation of several calcium dependent pathways that include proteases and nucleases. Reperfusion aggravates the cerebral parenchyma damage by forming free radicals that damage the membranes and proteins. Further, the opening of mitochondrial permeability transition pore releases various proapoptotic molecules that activate apoptotic cell death in cerebral parenchyma. In intracerebral hemorrhage the initial bleed causes physical disruption of the cellular architecture of the brain and the mass of the haematoma may increase intracranial pressure which can compress brain regions, increasing neuronal death result of necrotic and apoptotic mechanisms depending on the severity of insult. Created with BioRender.com.

induced brain injury by stroke, various mechanisms in the neurovascular bed can trigger oxidative stress, including mitochondrial dysfunction, increase vasogenic mediators, glutamate release, and depletion of antioxidant defense system. Mitochondria are both important intracellular organelles for energy metabolism organelles, the main intracellular source of ROS, and important targets for I/R brain injury [79]. During stroke, inflammatory cytokines, oxidative stress, and Ca^{+2} overload stimulate the mitochondria, inducing the production of higher ROS levels, thereby triggering the mitochondrial necrosis pathway and leading to cell death [80]. In addition, endothelial cells, and immune cells produce large amounts of ROS during the cerebral ischemia phase, which in turn induce the activation of nuclear factor-κappa B (NF-κB), inducible endothelial nitric oxide (iNOS), and proinflammatory factors, triggering the upregulation of vascular endothelial cell adhesion molecules and causing BBB permeability [81].

4.1.2 Intracerebral hemorraghe

Between 10 and 15% of all strokes in the USA are intracerebral hemorrhages, which has higher morbidity and mortality when compared to ischemic strokes. Multiple etiologies are associated with ICH, hypertension being the more common, followed by amyloid pathology, especially in older populations, vascular malformations, and coagulopathies [72]. After the initial bleed, there can be a continuous bleed for the next 24 hours, the so-called hematoma expansion. A delayed vascular disruption occurs after the first 24 hours; this includes BBB dysfunction, which can associate with edema formation and an influx of leukocytes into the brain parenchyma [82].

The role of ischemia in ICH-induced brain injury is controversial, as a reduction in blood flow may be a result rather than the cause of brain damage. This suggests that BBB's increased permeability is due to the direct effect of certain blood components (thrombin, fibrin, and hemoglobin, iron) or to the inflammatory response to these components [72]. This phenomenon may include further peripheral cell

infiltration and microglia activation, which may promote the higher secretion of proinflammatory cytokines and the activation of MMPs, as previously described in ischemic stroke [83].

4.2 Alteration of BBB by neurological disorders

4.2.1 Alzheimer's disease

Alzheimer's disease (AD) pathological hallmark is the accumulation of amyloid beta plaque deposits, which suggests the imbalance between its production and clearance rates may be due to a leaky BBB. The BBB dysfunction itself can also promote and accelerate the process of further AB production [84]. Diminished expression and dysfunction of ABC transporters at the BBB have been found in AD mice models [85], and two crucial BBB transporters in Abeta BBB's flow dynamics, p-glycoprotein LRP1, and RAGE have been identified as functionally impaired in AD. Expression of LRP1, which is in charge of the efflux of brain-derived Ab into blood across the BBB, is remarkably low at the BBB in AD patients' and AD models' brains [86]. Verapamil-PET studies in patients with mild AD, an exam that clinically assesses p-glycoprotein function, have found reduced activity of this transport in frontal, posterior cingulate, and the parietooccipital cortices, as well as in the hippocampus [87]. RAGE is a vital transporter that regulates the influx of circulating soluble ab intro the brain, which may promote neuroinflammation. Patients with AD develop increased levels of this transporter receptor both in brain endothelium and mural cells of the BBB [88].

There is enough evidence that associates AD with vascular disease at a pathological level [89]. Cerebral vessel pathology is not only a significant risk factor for AD but can also cause BBB disruption, as is the case with cerebral amyloid angiopathy [90]. Furthermore, changes in vascular biomarkers have been observed in preclinical AD before the development of cognitive impairment, and even before increases in routine AD biomarkers [91]. These findings support the two-hit vascular hypothesis of AD suggests that BBB dysfunction and brain hypoperfusion secondary to blood vessel damage may be the first hit that leads to ab accumulation and neuronal injury [92]. There is also evidence that at least two out of three BBB's main three cell lines are significantly compromised in AD. Accelerated pericyte degeneration and BBB breakdown is a distinguishing characteristic of AD-ApoE4 carriers mouse models [93]. On the other hand, astrocytic dysfunction, which has also been seen in AD models [84], may explain the hyperactivity of RAGE and hypoactivity of LRP1 in these patients' BBB. The pericyte degeneration initiates multiple pathways of neurodegeneration owing to the entry of several neurotoxic blood-derived proteins, including plasminogen, thrombin and fibrinogen which enter different areas of the CNS [93]. Plasmin, which is generated from circulating plasminogen, degrades the neuronal matrix protein laminin, thereby promoting neuronal injury. High concentrations of thrombin mediate neurotoxicity and memory impairment and accelerate BBB disruption [94].

4.2.2 Parkinson's disease

PD is one of the most prevalent neurodegenerative diseases after AD. It is characterized by filamentous and oligomeric α-synuclein (α-syn) accumulation, and degeneration of dopaminergic neurons in the substantia nigra leading to motor impairments [34]. Ever since the publication of Braak et al. studies, there is consensus that Parkinson's disease starts in the peripheral system and reaches the central nervous system in a retrograde (axon terminal to soma) spread of Lewy pathology.

Some authors have suggested that this spread could be through a hematogenous pathway [95]. Although PD patients in Braak stage 1 have their axon terminals outside the BBB, this same structure protects the somas of those axons, which reside in the central nervous system. Cerebrovascular disease also plays a part in PD, as both vascular disease and vascular risk factors aggravate motor and cognitive symptoms [96]. This may explain the BBB leakiness observed in these patients, as a recent study observed in the post-commissural putamen of PD patients, using histologic markers of serum protein, iron, and erythrocyte extravasation [97]. Regarding the extravasation of molecules through the cerebral vascular system, the histological analysis of PD patients reveals BBB breakdown in the striatum as shown by capillary leakages and accumulation of perivascular fibrinogen, immunoglobulins deposits, hemosiderin, red blood cells extravasation and leukocyte infiltration [98]. Increased BBB permeability and inducing inflammatory and necrotic processes in the brain parenchyma.

In patients with PD, a dysregulation of the transport systems has also been observed in the BBB, recent studies reveal that α-syn crosses the BBB, which could signify an important contributory event in PD pathogenesis (neurodegeneration) [99]. The α-syn oligomers crossed the BBB into the brain, in parenchyma where α-syn amplification and strain-specific pathology and neurotoxic phenotypes. In the other hand, regarding the clearance of the α-syn, this molecule is capable of inhibiting Aβ efflux suggesting and the endothelial LRP1 is a only potential efflux transporter for α-syn, however, LRP1 is similarly downregulated in PD [100], this could result in impaired α-syn BBB clearance and accumulation in brain, suggesting that the high levels of α-syn produced peripherally can enter the brain in the presence of BBB breakdown, which may also contribute to development of PD pathology.

4.2.3 Multiple sclerosis

Multiple sclerosis (MS) is an autoimmune disease with an early BBB disruption pattern. One clear indication is the presence of Gadolinium-enhancing lesions on magnetic resonance, which translates in extravasation of intravascular contrast due to brain parenchyma and its associated active inflammation. Moreover, an increasing amount of evidence shows this disruption could not be restricted to Gd-enhancing lesions, as observed in non-enhancing areas in postmortem MS brains [101]. As the entry of inflammatory infiltrate occurs in the brain's perivascular regions, it is intuitive to think BBB disruption is very likely an early event in lesion formation.

There is also evidence of maladaptive changes in the NVU's components. One great example is ECs, which upregulate adhesion molecules and display chemokines on their luminal surface, to promote transcellular immune cell migration [102]. Also, in MS, TJ abnormalities can be seen, as one study observed abnormal ZO-1 at TJs in sections of primary progressive MS patients' cortical grey matter [101]. The BM in these patients' lesions also appears discontinuous. MRI studies have shown hypoperfusion in early and advanced stages of MS, suggesting the presence of metabolic injury in the brain parenchyma in a hypoxia-like fashion [103]. Regarding a primary BBB dysfunction, studies have focused on astrocytes and pericytes, whose maladaptive changes could explain the reduction in capillary blood flow and further hypoxia. D'haeseleer et al. observed that the hypoperfusion in MS could be mediated by astrocyte's released endothelin-1 (ET-1), as it can be normalized with an ET-1 antagonist [104]. This body of evidence conveys heterogeneous pathophysiology in MS, one that included BBB breakdown as a primary event and not only as a secondary consequence [105].

5. Conclusion and future directions

The relevance of the NVU in the support of cerebral homeostasis of the BBB is being partly established with recent evidence. The multifactorial interactions between their components are extremely refined, expressing the complexity of the central CNS physiology. The knowledge of each of the components and their respective pathways are critical to understanding various neurovascular diseases, such as cerebrovascular injury (e.g. stroke) and neurological disorders (e.g. Alzheimer's). Although despite current knowledge, many questions about the role of each component NVU, pathways and crosstalking still have no answer. These advances have uncovered gaps in our knowledge of neurovascular health and have provided us with the roadmap to ask new questions that should be addressed by the future studies. Finally, based on the current state of our knowledge, it is probably time to think about BBB not only as an impermeable cellular membrane which protects brain from peripheral influences and should be breached for therapeutic CNS drug delivery, but also as an enormous source of understudied molecular and cellular targets in the pathophysiological states, which if explored could change the paradigm about brain diseases therapy and could lead to development of novel BBB-based personalized approaches to treat them.

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References

[1] Tumani H, Huss A, Bachhuber F. The cerebrospinal fluid and barriers anatomic and physiologic considerations. Handbook of Clinical Neurology. 2017;**146**:21-32. DOI: 10.1016/B978-0-12-804279-3.00002-2

[2] Pandit R, Chen L, Götz J. The blood-brain barrier: Physiology and strategies for drug delivery [published online ahead of print, 2019 Nov 29]. Adv Drug Deliv Rev. 2019; S0169-409X (19)30238-8. doi: 10.1016/j.addr.2019.11.009.

[3] Liebner S, Dijkhuizen RM, Reiss Y, Plate KH, Agalliu D, Constantin G. Functional morphology of the blood-brain barrier in health and disease. Acta Neuropathologica. 2018;**135**(3):311-336. DOI: 10.1007/s00401-018-1815-1

[4] Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. Nature Medicine. 2013;**19**(12):1584-1596. DOI: 10.1038/nm.3407

[5] Iadecola C. The Neurovascular Unit Coming of Age: A Journey through Neurovascular Coupling in Health and Disease. Neuron. 2017;**96**(1):17-42. DOI: 10.1016/j.neuron.2017.07.030

[6] Phillips AA, Chan FH, Zheng MM, Krassioukov AV, Ainslie PN. Neurovascular coupling in humans: Physiology, methodological advances and clinical implications. Journal of Cerebral Blood Flow and Metabolism. 2016;**36**(4):647-664. DOI: 10.1177/0271678X15617954

[7] Filosa JA, Morrison HW, Iddings JA, Du W, Kim KJ. Beyond neurovascular coupling, role of astrocytes in the regulation of vascular tone. Neuroscience. 2016;**323**:96-109. DOI: 10.1016/j.neuroscience.2015.03.064

[8] Engelhardt B, Sorokin L. The blood-brain and the blood-cerebrospinal fluid barriers: function and dysfunction. Seminars in Immunopathology. 2009;**31**(4):497-511. DOI: 10.1007/s00281-009-0177-0

[9] Goldwaser EL, Acharya NK, Sarkar A, Godsey G, Nagele RG. Breakdown of the Cerebrovasculature and Blood-Brain Barrier: A Mechanistic Link Between Diabetes Mellitus and Alzheimer's Disease. Journal of Alzheimer's Disease. 2016;**54**(2):445-456. DOI: 10.3233/JAD-160284

[10] Langen UH, Ayloo S, Gu C. Development and Cell Biology of the Blood-Brain Barrier. Annual Review of Cell and Developmental Biology. 2019;**35**:591-613. DOI: 10.1146/annurev-cellbio-100617-062608

[11] Muoio V, Persson PB, Sendeski MM. The neurovascular unit concept review. Acta Physiologica (Oxford, England). 2014;**210**(4):790-798. DOI: 10.1111/apha.12250

[12] Dejana E, Giampietro C. Vascular endothelial-cadherin and vascular stability. Current Opinion in Hematology. 2012;**19**(3):218-223. DOI: 10.1097/MOH.0b013e3283523e1c

[13] Tietz S, Engelhardt B. Brain barriers: Crosstalk between complex tight junctions and adherens junctions. The Journal of Cell Biology. 2015;**209**(4):493-506. DOI: 10.1083/jcb.201412147

[14] Blanchette M, Daneman R. Formation and maintenance of the BBB. Mechanisms of Development. 2015;**138**(Pt 1):8-16. DOI: 10.1016/j.mod.2015.07.007

[15] Obermeier B, Verma A, Ransohoff RM. The blood-brain barrier. Handbook of Clinical Neurology.

2016;**133**:39-59. DOI: 10.1016/B978-0-444-63432-0.00003-7

[16] Brown LS, Foster CG, Courtney JM, King NE, Howells DW, Sutherland BA. Pericytes and Neurovascular Function in the Healthy and Diseased Brain. Front Cell Neurosci. 2019; 13:282. Published 2019 Jun 28. doi:10.3389/fncel.2019.00282.

[17] Mathiisen TM, Lehre KP, Danbolt NC, Ottersen OP. The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction. Glia. 2010;**58**(9):1094-1103. DOI: 10.1002/glia.20990

[18] Peppiatt CM, Howarth C, Mobbs P, Attwell D. Bidirectional control of CNS capillary diameter by pericytes. Nature. 2006;**443**(7112):700-704. DOI: 10.1038/nature05193

[19] Hill J, Rom S, Ramirez SH, Persidsky Y. Emerging roles of pericytes in the regulation of the neurovascular unit in health and disease. Journal of Neuroimmune Pharmacology. 2014;**9**(5):591-605. DOI: 10.1007/s11481-014-9557-x

[20] Keaney J, Campbell M. The dynamic blood-brain barrier. The FEBS Journal. 2015;**282**(21):4067-4079. DOI: 10.1111/febs.13412

[21] Armulik A, Genové G, Mäe M, et al. Pericytes regulate the blood-brain barrier. Nature. 2010;**468**(7323):557-561. DOI: 10.1038/nature09522

[22] Nuriya M, Hirase H. Involvement of astrocytes in neurovascular communication. Progress in Brain Research. 2016;**225**:41-62. DOI: 10.1016/bs.pbr.2016.02.001

[23] Tait MJ, Saadoun S, Bell BA, Papadopoulos MC. Water movements in the brain: role of aquaporins. Trends in Neurosciences. 2008;**31**:37-43. DOI: 10.1016/j.tins.2007.11.003

[24] WosikK,CayrolR,Dodelet-DevillersA, Berthelet F, Bernard M, Moumdjian R, et al. Angiotensin II controls occludin function and is required for blood-brain barrier maintenance: relevance to multiple sclerosis. The Journal of Neuroscience. 2007;**27**:9032-9042. DOI: 10.1523/JNEUROSCI.2088-07.2007

[25] Bell RD, Winkler EA, Singh I, Sagare AP, Deane R, Wu Z, et al. Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. Nature. 2012;**485**:512-551. DOI: 10.1038/nature11087

[26] Daneman R, Prat A. The blood-brain barrier. Cold Spring Harb Perspect Biol. 2015;7(1): a020412. Published 2015 Jan 5. doi:10.1101/cshperspect.a020412.

[27] Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science. 2010;**330**:841-845. DOI: 10.1126/science.1194637

[28] Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhozhij S, et al. Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. Blood. 2010;**116**:829-840. DOI: 10.1182/blood-2009-12-257832

[29] Ju F, Ran Y, Zhu L, Cheng X, Gao H, Xi X, et al. Increased BBB Permeability Enhances Activation of Microglia and Exacerbates Loss of Dendritic Spines After Transient Global Cerebral Ischemia. Frontiers in Cellular Neuroscience. 2018;**12**:236. DOI: 10.3389/fncel.2018.00236

[30] Yin X, Wright J, Wall T, Grammas P. Brain endothelial cells synthesize neurotoxic thrombin in Alzheimer's disease. The American

Journal of Pathology. 2010;**176**:1600-1606. DOI: 10.2353/ajpath.2010.090406

[31] Thomsen MS, Routhe LJ, Moos T. The vascular basement membrane in the healthy and pathological brain. Journal of Cerebral Blood Flow and Metabolism. 2017;**37**(10):3300-3317. DOI: 10.1177/0271678X17722436

[32] Xu L, Nirwane A, Yao Y. Basement membrane and blood-brain barrier. Stroke Vasc Neurol. 2018;**4**(2):78-82. DOI: 10.1136/svn-2018-000198

[33] Leybaert L. Neurobarrier coupling in the brain: a partner of neurovascular and neurometabolic coupling? Journal of Cerebral Blood Flow and Metabolism. 2005;**25**(1):2-16. DOI: 10.1038/sj.jcbfm.9600001

[34] Sweeney MD, Zhao Z, Montagne A, Nelson AR, Zlokovic BV. Blood-Brain Barrier: From Physiology to Disease and Back. Physiological Reviews. 2019;**99**(1):21-78. DOI: 10.1152/physrev.00050.2017

[35] Ballabh P, Braun A, Nedergaard M. The blood-brain barrier: an overview: structure, regulation, and clinical implications. Neurobiology of Disease. 2004;**16**(1):1-13. DOI: 10.1016/j.nbd.2003.12.016

[36] Greene C, Campbell M. Tight junction modulation of the blood brain barrier: CNS delivery of small molecules. Tissue Barriers. 2016;**4**(1):e1138017. DOI: 10.1080/21688370.2015.1138017

[37] Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. The Journal of Cell Biology. 1998;**141**:1539-1550. DOI: 10.1083/jcb.141.7.1539

[38] Nitta T, Hata M, Gotoh S, Seo Y, Sasaki H, Hashimoto N, et al. Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. The Journal of Cell Biology. 2003;**161**:653-660. DOI: 10.1083/jcb.200302070

[39] Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron. 2008;**57**:178-201. DOI: 10.1016/j.neuron.2008.01.003

[40] Van Itallie CM, Anderson JM. Architecture of tight junctions and principles of molecular composition. Seminars in Cell & Developmental Biology. 2014;**36**:157-165. DOI: 10.1016/j.semcdb.2014. 08.011

[41] Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nature Reviews. Neuroscience. 2011;**12**:723-738. DOI: 10.1038/nrn3114

[42] Reinhold AK, Rittner HL. Barrier function in the peripheral and central nervous system-a review. Pflügers Archiv. 2017;**469**(1):123-134. DOI: 10.1007/s00424-016-1920-8

[43] Dejana E, Lampugnani MG, Martinez-Estrada O, Bazzoni G. The molecular organization of endothelial junctions and their functional role in vascular morphogenesis and permeability. The International Journal of Developmental Biology. 2000;**44**:743-748

[44] Hawkins BT, Davis TP. The blood-brain barrier/neurovascular unit in health and disease. Pharmacological Reviews. 2005;**57**(2):173-185. DOI: 10.1124/pr.57.2.4

[45] Nagasawa K, Chiba H, Fujita H, Kojima T, Saito T, Endo T, et al. Possible involvement of gap junctions in the barrier function of tight junctions of brain and lung endothelial cells. Journal of Cellular Physiology. 2006;**208**:123-132. DOI: 10.1002/jcp.20647

[46] Nielsen MS, Axelsen LN, Sorgen PL, Verma V, Delmar M, Holstein-Rathlou NH. Gap junctions. Comprehensive Physiology. 2012;**2**(3):1981-2035. DOI: 10.1002/cphy.c110051

[47] Erdő F, Krajcsi P. Age-Related Functional and Expressional Changes in Efflux Pathways at the Blood-Brain Barrier. Frontiers in Aging Neuroscience. 2019;**11**:196. DOI: 10.3389/fnagi.2019.00196

[48] Jurcovicova J. Glucose transport in brain effect of inflammation. Endocrine Regulations. 2014;**48**(1):35-48. DOI: 10.4149/endo_2014_01_35

[49] Winkler EA, Nishida Y, Sagare AP, Rege SV, Bell RD, Perlmutter D, et al. GLUT1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration. Nature Neuroscience. 2015;**18**:521-530. DOI: 10.1038/nn.3966

[50] O'Kane RL, Hawkins RA. Na+ dependent transport of large neutral amino acids occurs at the abluminal membrane of the blood-brain barrier. American Journal of Physiology. Endocrinology and Metabolism. 2003;**285**:E1167-E1173. DOI: 10.1152/ajpendo.00193.2003

[51] Mann GE, Yudilevich DL, Sobrevia L. Regulation of amino acid and glucose transporters in endothelial and smooth muscle cells. Physiol Rev. 2003; 83: 183-252. doi:10. 1152/physrev.00022.2002.

[52] O'Kane RL, Martínez-López I, De Joseph MR, Viña JR, Hawkins RA. Na (+)-dependent glutamate transporters (EAAT1, EAAT2, and EAAT3) of the blood-brain barrier. A mechanism for glutamate removal. The Journal of Biological Chemistry. 1999;**274**:31891-31895. DOI: 10.1074/ jbc.274.45.31891

[53] Lee WJ, Hawkins RA, Viña JR, Peterson DR. Glutamine transport by the blood-brain barrier: a possible mechanism for nitrogen removal. American Journal of Physiology. Cell Physiology. 1998;**274**:C1101-C1107. DOI: 10.1152/ajpcell.1998.274.4.C1101

[54] Simpson IA, Carruthers A, Vannucci SJ. Supply and demand in cerebral energy metabolism: the role of nutrient transporters. Journal of Cerebral Blood Flow and Metabolism. 2007;**27**:1766-1791. DOI: 10.1038/sj.jcbfm.9600521

[55] Cass CE, Young JD, Baldwin SA. Recent advances in the molecular biology of nucleoside transporters of mammalian cells. Biochem Cell Biol. 1998; 76: 761-770. doi:10. 1139/o98-095.

[56] Jefferies WA, Brandon MR, Hunt SV, Williams AF, Gatter KC, Mason DY. Transferrin receptor on endothelium of brain capillaries. Nature. 1984; 312: 162-163. doi:10. 1038/312162a0.

[57] to S, Yanai M, Yamaguchi S, Couraud PO, Ohtsuki S. Regulation of Tight-Junction Integrity by Insulin in an in Vitro Model of Human Blood-Brain Barrier. J Pharm Sci. 2017; 106: 2599-2605. doi: 10.1016/j.xphs.2017.04.036.

[58] Golden PL, Maccagnan TJ, Pardridge WM. Human blood-brain barrier leptin receptor. Binding and endocytosis in isolated human brain microvessels. The Journal of Clinical Investigation. 1997;**99**:14-18. DOI: 10.1172/JCI119125

[59] Pardridge WM. Drug transport across the blood-brain barrier. Journal of Cerebral Blood Flow and Metabolism. 2012;**32**:1959-1972. DOI: 10.1038/jcbfm.2012.126

[60] Deane R, Singh I, Sagare AP, Bell RD, Ross NT, La Rue B, et al. A multimodal RAGE-specific inhibitor reduces amyloid-mediated brain disorder in a mouse model of Alzheimer disease. The Journal of Clinical

Investigation. 2012;**122**:1377-1392. DOI: 10.1172/ JCI58642

[61] Funck VR, Ribeiro LR, Pereira LM, de Oliveira CV, Grigoletto J, Della-Pace ID, et al. Contrasting effects of Na, K - ATPase activation on seizure activity in acute versus chronic models. Neuroscience. 2015;**298**:171-179. DOI: 10.1016/j.neuroscience.2015.04.031

[62] Taylor CJ, Nicola PA, Wang S, Barrand MA, Hladky SB. Transporters involved in regulation of intracellular pH in primary cultured rat brain endothelial cells. The Journal of Physiology. 2006;**576**:769-785. DOI: 10.1113/jphysiol.2006.117374

[63] Sonkusare SK, Bonev AD, Ledoux J, Liedtke W, Kotlikoff MI, Heppner TJ, Hill-Eu- banks DC, Nelson MT. Elementary Ca2 signals through endothelial TRPV4 channels regulate vascular function. Science 2012; 336: 597-601. doi:10.1126/science. 1216283.

[64] Jia SW, Liu XY, Wang SC, Wang YF. Vasopressin Hypersecretion-Associated Brain Edema Formation in Ischemic Stroke: Underlying Mechanisms. Journal of Stroke and Cerebrovascular Diseases. 2016;**25**(6):1289-1300. DOI: 10.1016/j. jstrokecerebrovasdis.2016.02.002

[65] Armulik A, Genové G, Mäe M, Nisancioglu MH, Wallgard E, Niaudet C, et al. Pericytes regulate the blood-brain barrier. Nature. 2010;**468**:557-561. DOI: 10.1038/nature09522

[66] Sagare AP, Bell RD, Zhao Z, Ma Q, Winkler EA, Ramanathan A, et al. Pericyte loss influences Alzheimer-like neurodegeneration in mice. Nature Communications. 2013;**4**:2932. DOI: 10.1038/ncomms3932

[67] Bell RD, Winkler EA, Singh I, Sagare AP, Deane R, Wu Z, et al. Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. Nature. 2012;**485**:512-516. DOI: 10.1038/nature11087

[68] Schenk GJ, de Vries HE. Altered blood-brain barrier transport in neuro-inflammatory disorders. Drug Discovery Today: Technologies. 2016;**20**:5-11. DOI: 10.1016/j.ddtec.2016.07.002

[69] Worzfeld T, Schwaninger M. Apicobasal polarity of brain endothelial cells. Journal of Cerebral Blood Flow and Metabolism. 2016 Feb;**36**(2):340-362. DOI: 10.1016/j.ddtec.2016.07.002

[70] Rosenberg GA. Neurological diseases in relation to the blood-brain barrier. Journal of Cerebral Blood Flow and Metabolism. 2012;**32**(7):1139-1151. DOI: 10.1038/jcbfm.2011.197

[71] O'Donnell ME. Blood-brain barrier Na transporters in ischemic stroke. Advances in Pharmacology. 2014;**71**:113-146. DOI: 10.1016/ bs.apha.2014.06.011

[72] Keep RF, Andjelkovic AV, Stamatovic SM, Shakui P, Ennis SR. Ischemia-induced endothelial cell dysfunction. Acta Neurochirurgica. Supplement. 2005;**95**:399-402. DOI: 10.1007/3-211-32318-x_81

[73] Yang Y, Estrada EY, Thompson JF, Liu W, Rosenberg GA. Matrix metalloproteinase-mediated disruption of tight junction proteins in cerebral vessels is reversed by synthetic matrix metalloproteinase inhibitor in focal ischemia in rat. Journal of Cerebral Blood Flow and Metabolism. 2007;**27**(4):697-709. DOI: 10.1038/ sj.jcbfm.9600375

[74] Abdullahi W, Tripathi D, Ronaldson PT. Blood-brain barrier dysfunction in ischemic stroke: targeting tight junctions and transporters for vascular protection. American Journal of Physiology. Cell Physiology. 2018;**315**(3):C343-C356. DOI: 10.1152/ajpcell.00095.2018

[75] Boutin H, LeFeuvre RA, Horai R, Asano M, Iwakura Y, Rothwell NJ. Role of IL-1alpha and IL-1beta in ischemic brain damage [published correction appears in J Neurosci 2001 Sep 1;21(17):1a]. The Journal of Neuroscience. 2001;**21**(15):5528-5534. DOI: 10.1523/JNEUROSCI.21-15-05528.2001

[76] Rochfort KD, Cummins PM. Cytokine-mediated dysregulation of zonula occludens-1 properties in human brain microvascular endothelium. Microvascular Research. 2015;**100**:48-53. DOI: 10.1016/j.mvr.2015.04.010

[77] Kassner A, Merali Z. Assessment of Blood-Brain Barrier Disruption in Stroke. Stroke. 2015;**46**(11):3310-3315. DOI: 10.1161/STROKEAHA.115.008861

[78] Carvalho AN, Firuzi O, Gama MJ, Horssen JV, Saso L. Oxidative Stress and Antioxidants in Neurological Diseases: Is There Still Hope? Current Drug Targets. 2017;**18**(6):705-718. DOI: 10.2174/1389450117666160401120514

[79] Zhao XY, Lu MH, Yuan DJ, Xu DE, Yao PP, Ji WL, et al. Mitochondrial Dysfunction in Neural Injury. Frontiers in Neuroscience. 2019;**4**:13-30. DOI: 10.3389/fnins.2019.00030

[80] Zhu Y, Wang H, Fang J, Dai W, Zhou J, Wang X, et al. SS-31 Provides Neuroprotection by Reversing Mitochondrial Dysfunction after Traumatic Brain Injury. Oxidative Medicine and Cellular Longevity. 2018;**27**:4783602. DOI: 10.1155/2018/4783602

[81] Abdul-Muneer PM, Chandra N, Haorah J. Interactions of oxidative stress and neurovascular inflammation in the pathogenesis of traumatic brain injury. Molecular Neurobiology. 2015;**51**(3):966-979. DOI: 10.1007/s12035-014-8752-3

[82] Murai Y, Ikeda Y, Teramoto A, Tsuji Y. Magnetic resonance imaging-documented extravasation as an indicator of acute hypertensive intracerebral hemorrhage. Journal of Neurosurgery. 1998;**88**(4):650-655. DOI: 10.3171/jns.1998.88.4.0650

[83] Moxon-Emre I, Schlichter LC. Neutrophil depletion reduces blood-brain barrier breakdown, axon injury, and inflammation after intracerebral hemorrhage. Journal of Neuropathology and Experimental Neurology. 2011;**70**(3):218-235. DOI: 10.1097/NEN.0b013e31820d94a5

[84] Cai Z, Qiao PF, Wan CQ, Cai M, Zhou NK, Li Q. Role of Blood-Brain Barrier in Alzheimer's Disease. Journal of Alzheimer's Disease. 2018;**63**(4):1223-1234. DOI: 10.3233/JAD-180098

[85] Cirrito JR, Deane R, Fagan AM, Spinner ML, Parsadanian M, Finn MB, et al. P-glycoprotein deficiency at the blood-brain barrier increases amyloid-beta deposition in an Alzheimer disease mouse model. The Journal of Clinical Investigation. 2005;**115**:3285-3290. DOI: 10.1172/ JCI25247

[86] Deane R, Bell RD, Sagare A, Zlokovic BV. Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease. CNS & Neurological Disorders Drug Targets. 2009;**8**(1):16-30. DOI: 10.2174/187152709787601867

[87] Deo AK, Borson S, Link JM, et al. Activity of P-Glycoprotein, a β-Amyloid Transporter at the Blood-Brain Barrier, Is Compromised in Patients with Mild Alzheimer Disease. Journal of Nuclear Medicine. 2014;**55**(7):1106-1111. DOI: 10.2967/jnumed.113.130161

[88] Donahue JE et al. RAGE, LRP-1, and amyloid-beta protein in Alzheimer's disease. Acta Neuropathol (Berl). 2006;**112**:405-415. DOI: 10.1007/s00401-006-0115-3

[89] Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain Infarction and the Clinical Expression of Alzheimer Disease: The Nun Study. Journal of the American Medical Association. 1997;**277**(10):813-817. DOI: 10.1001/jama.1997.03540340047031

[90] Saito S, Ihara M. Interaction between cerebrovascular disease and Alzheimer pathology. Current Opinion in Psychiatry. 2016;**29**(2):168-173. DOI: 10.1097/YCO.0000000000000239

[91] Iturria-Medina Y, Sotero RC, Toussaint PJ, Mateos-Pérez JM, Evans AC; Alzheimer's Disease Neuroimaging Initiative. Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. Nat Commun. 2016; 7:11934. doi: 10.1038/ncomms11934.

[92] Nelson AR, Sweeney MD, Sagare AP, Zlokovic BV. Neurovascular dysfunction and neurodegeneration in dementia and Alzheimer's disease. Biochimica et Biophysica Acta. 2016;**1862**(5):887-900. DOI: 10.1016/j.bbadis.2015.12.016

[93] Halliday MR, Rege SV, Ma Q, Zhao Z, Miller CA, Winkler EA, et al. Accelerated pericyte degeneration and blood-brain barrier breakdown in apolipoprotein E4 carriers with Alzheimer's disease. Journal of Cerebral Blood Flow and Metabolism. 2016;**36**:216-227. DOI: 10.1038/jcbfm.2015.44

[94] Sweeney M, Sagare A, Zlokovic B. Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol. 2018; 14: 133-150. doi.org/10.1038/nrneurol.2017.188.

[95] Woulfe JM, Gray MT, Gray DA, Munoz DG, Middeldorp JM. Hypothesis: a role for EBV-induced molecular mimicry in Parkinson's disease. Parkinsonism & Related Disorders. 2014;**20**:685-694. DOI: 10.1016/j.parkreldis.2014.02.031

[96] Malek N, Lawton MA, Swallow DM, et al. Vascular disease and vascular risk factors in relation to motor features and cognition in early Parkinson's disease. Movement Disorders. 2016;**31**(10):1518-1526. DOI: 10.1002/mds.26698

[97] Gray MT, Woulfe JM. Striatal blood-brain barrier permeability in Parkinson's disease. Journal of Cerebral Blood Flow and Metabolism. 2015;**35**(5):747-750. DOI: 10.1038/jcbfm.2015.32

[98] Chung YC, Kim Y-S, Bok E, Yune TY, Maeng S, Jin BK. MMP-3 contributes to nigrostriatal dopaminergic neuronal loss, BBB damage, and neuroinflammation in an MPTP mouse model of Parkinson's disease. Mediators Inflamm. 2013; 370526. doi:10. 1155/2013/370526.

[99] Peelaerts W, Bousset L, Van der Perren A, Moskalyuk A, Pulizzi R, Giugliano M, Van den Haute C, Melki R, Baekelandt V. Synuclein strains cause distinct synucleinopathies after local and systemic administration. Nature. 2015; 522: 340-344. doi:10. 1038/nature14547.

[100] Sui Y-T, Bullock KM, Erickson MA, Zhang J, Banks WA. Alpha synuclein is transported into and out of the brain by the blood-brain barrier. Peptides. 2014;**62**:197-202. DOI: 10.1016/j.peptides.2014.09.018

[101] Vos CM, Geurts JJ, Montagne L, van Haastert ES, Bö L, van der Valk P, et al. Blood-brain barrier alterations in both focal and diffuse abnormalities on postmortem MRI in multiple sclerosis. Neurobiology of Disease. 2005;**20**(3):953-960. DOI: 10.1016/j.nbd.2005.06.012

[102] Alvarez JI, Cayrol R, Prat A. Disruption of central nervous system barriers in multiple sclerosis. Biochimica

et Biophysica Acta. 2011;**1812**(2):252-264. DOI: 10.1016/j.bbadis.2010.06.017

[103] Spencer JI, Bell JS, DeLuca GC. Vascular pathology in multiple sclerosis: reframing pathogenesis around the blood-brain barrier. Journal of Neurology, Neurosurgery, and Psychiatry. 2018;**89**(1):42-52. DOI: 10.1136/jnnp-2017-316011

[104] D'haeseleer M, Beelen R, Fierens Y, Cambron M, Vanbinst AM, Verborgh C, et al. Cerebral hypoperfusion in multiple sclerosis is reversible and mediated by endothelin-1. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**(14):5654-5658. DOI: 10.1073/pnas.1222560110

[105] Keep RF, Zhou N, Xiang J, Andjelkovic AV, Hua Y, Xi G. Vascular disruption and blood-brain barrier dysfunction in intracerebral hemorrhage. Fluids Barriers CNS. 2014;**11**:18. DOI: 10.1186/2045-8118-11-18