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REVIEW



# Basic physiology of the blood-brain barrier in health and disease: a brief overview

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**ABSTRACT**

The blood-brain barrier (BBB), a dynamic interface between blood and brain constituted mainly by endothelial cells of brain microvessels, robustly restricts the entry of potentially harmful blood-sourced substances and cells into the brain, however, many therapeutically active agents concurrently cannot gain access into the brain at effective doses in the presence of an intact barrier. On the other hand, breakdown of BBB integrity may involve in the pathogenesis of various neurodegenerative diseases. Besides, certain diseases/disorders such as Alzheimer's disease, hypertension, and epilepsy are associated with varying degrees of BBB disruption. In this review, we aim to highlight the current knowledge on the cellular and molecular composition of the BBB with special emphasis on the major transport pathways across the barrier type endothelial cells. We further provide a discussion on the innovative brain drug delivery strategies in which the obstacle formed by BBB interferes with effective pharmacological treatment of neurodegenerative diseases/disorders.

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## Introduction

The brain is the most critical organ that controls body systems in humans. Oxygen and nutrients, mainly glucose and amino acids, are supplied to the cells in the brain parenchyma by an elaborate network of blood capillaries. The estimated total length of brain microvessels is about 600–700 km and the total area of the endothelial surface in brain vasculature including capillaries, venules, arterioles, veins, and arteries approximates 20 $m^2$.[1,2] The brain is extremely sensitive to a wide range of potentially toxic substances in circulation, and the proper neuronal function necessitates an optimal microenvironment that is controlled and regulated by three different barrier systems; the blood-brain barrier (BBB) formed by brain microvessel endothelial cells (Figure 1), the blood-cerebrospinal fluid barrier (BCSFB) formed by choroid plexus epithelial cells (Figure 2Figure 3), and the meningeal barrier formed by arachnoid epithelial cells.[3–5] It is suggested that neuronal homeostasis within the brain parenchyma is mainly regulated by the BBB since the total area of the luminal surface with BBB activity is estimated to be about 1000 times larger than that with BCSFB.[6]

The concept of the BBB was first established in the late 19$^{th}$ century by Paul Ehrlich, who observed that trypan blue dye injected into the rat circulation resulted in the staining of peripheral organs but not the brain and spinal cord. In the following years, Goldman injected trypan blue into the cerebrospinal fluid (CSF), and he demonstrated that the staining was only restricted to the central nervous system (CNS), but not to the other body tissues.[7] By the advent of electron microscopy and its widespread use in the evaluation of biological tissues in the 1960s, the presence of the BBB was confirmed using an electron-dense tracer, horseradish peroxidase (40 kD), which had been observed to pass through the vascular endothelium in peripheral tissues in contrast to that in the brain.[8]

The BBB, as a dynamic regulatory interface between blood and brain, protects neuronal microenvironment required for the proper functioning of neuronal circuits, synaptic transmission and remodeling, angiogenesis, and neurogenesis, by constantly controlling trafficking of molecules and preventing circulatory immune cell entry into the brain via paracellular and transcellular pathways.[3,9–12] The access of certain blood-borne

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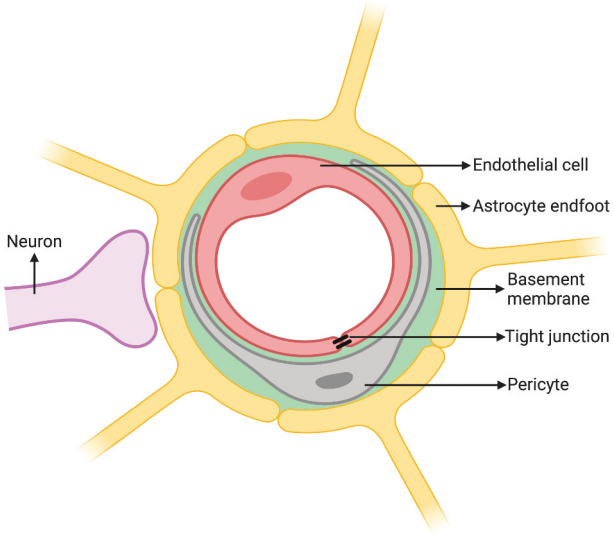


**Figure 1.** Schematic drawing of the BBB constituted by barrier type endothelial cell with TJ sharing the basement membrane with pericyte and the surrounding astrocyte endfeet. Created with BioRender.com.

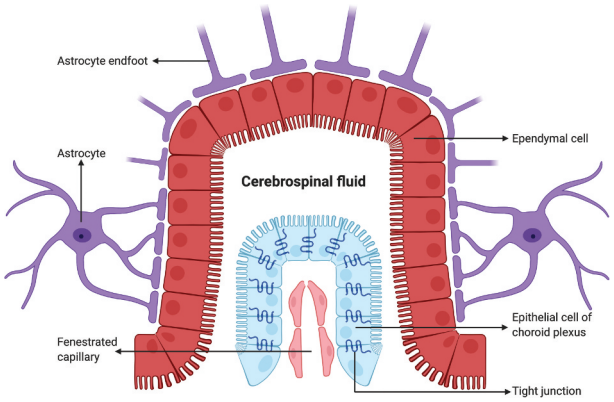


**Figure 2.** Schematic drawing of the BCSFB constituted by epithelial cells of choroid plexus with TJs, which secrete CSF derived from plasma in blood capillary without barrier properties into the ventricular space lined with ependymal cells. Created with BioRender.com.

neuroactive solutes, such as glutamate, glycine, norepinephrine, epinephrine, and peptide hormones into the brain is also significantly limited by the action BBB.[13–15] A healthy BBB not only protects the neurons but also is crucial for the physiologic functions of glial cells and pericytes.

The afore-mentioned protective activities of BBB brings with a concomitant obstacle for the access of therapeutic agents into the brain at effective doses for the treatment of neurodegenerative diseases. The presence of an intact BBB excludes approximately 98% of small molecule

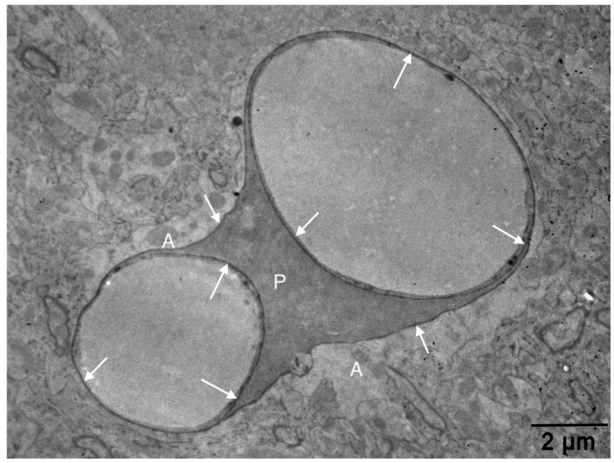


**Figure 3.** An electron micrograph from our image archive showing a pericyte (p) partly investing the endothelial cells of blood capillaries. Note that both endothelial cells and the pericyte are embedded in the basement membrane marked by extravasated electron-dense horseradish peroxidase tracer accumulation (arrows) owing to BBB disruption. A: astrocyte endfoot.

drugs and nearly all large therapeutics, such as recombinant peptides, proteins, anti-sense-agents, and genetic vectors from the brain.[16] Moreover, the fraction of therapeutic antibodies such as immunoglobulin G that reaches into the brain parenchyma following intravenous administration is estimated to be as low as 0.1%.[17] On the other hand, certain systemic diseases or CNS disorders are likely to evoke alterations in BBB integrity, leading to BBB disruption and loss of neuronal homeostasis.[18–21]

In this review, we present an overview of the structure and function of the BBB in both healthy and pathological conditions, the alterations in BBB integrity associated with neurodegenerative disorders/diseases and novel strategies to enhance targeted drug delivery into the brain.

## Neurovascular unit

In the last 2 decades, the term "neurovascular unit" has started to be used to define a well-structured complex that is involved in the development and maintenance of BBB integrity and in the regulation of cerebral blood flow. The major constituents of the unit are barrier type endothelial cells interacting with the basement membrane, pericytes, vascular smooth muscle cells, astrocytes, microglia, oligodendroglia, and neurons.[22–26]

## Barrier type endothelial cells

The cellular structures that lie in the interface between the blood and the brain are endothelial cells, pericytes, and astrocyte endfeet, which along with a cellular product, basement membrane, collectively compose the major constituents of the BBB and orchestrate the trafficking of molecules and cells between the two compartments. The primary part of the BBB is made up of capillaries that have luminal diameters of less than 10 μm.[27,28] In contrast to the various types of peripheral capillaries with different capacities of permeability found in other organs, brain capillaries display barrier type endothelial cells with a variety of distinguished properties, which render them the chief element of BBB structure and the main actor in the maintenance of neuronal homeostasis. Accumulated data show that brain capillary endothelial cells are sealed with tight junctions (TJs), which restrict the paracellular transport, and lack endothelial fenestrations and possess relatively few caveolar vesicles which limit the transcellular transport.[3,29,30] In addition, barrier type endothelial cells exhibit specific transport and carrier proteins that are located both in the luminal and abluminal plasma membranes.[31–33] Moreover, these cells are also considered to form an endocrine secretory tissue that produces a variety of local hormones such as nitric oxide, prostaglandins, and cytokines.[34–36] A continuous basement membrane, pericytes and astrocyte endfeet surrounding the endothelium provide anatomical support to the BBB.[3,37,38]

### *Physical barrier (Tight junctions)*

Barrier type endothelial cells are normally adjoined to each other by TJs and adherens junctions, which collectively constitute a physical barrier that limits the paracellular pathway.[22] The exchange of polar substances between the blood and the brain is strictly controlled and significantly reduced by the TJ proteins.[3,39]

### *Metabolic barrier*

The brain capillary endothelial cells express a variety of enzymes including γ-glutamyl transpeptidase, alkaline phosphatase, aromatic acid decarboxylase, monoamine oxidase, and cytochrome P450, which metabolize and inactivate molecules such as neuroactive and neurotoxic compounds, peptides, and ATP.[6,36,40–43] Barrier type endothelial cells also possess certain crucial transporters such as Na+-K+ ATPase, and glucose transporter (Glut)-1 which take part in the regulation of the composition of neuronal microenvironment.[44,45]

### *Efflux barrier*

The barrier type endothelial cells of the brain capillaries express certain efflux transporters, designated as multidrug-resistance transporters localized predominantly on the luminal plasma membrane.[46,47] These transporters include *P*-gp, also called mdr-1, and breast cancer resistance protein which extrude the administered xenobiotics, compounds that include lipophilic and cationic drugs, from the brain capillary endothelial cells back to the circulation and hence reduce the delivery of drugs into the brain parenchyma at effective doses thereby posing an obstacle to the treatment of neurodegenerative disorders/diseases. A number of chemical compounds and chemotherapeutic agents used in daily clinical practice have been described as the substrates of these transporters.[48,49] Successful management of tumors and refractory epilepsy is reported to be overwhelmed by the activity of *P*-gp.[50,51] Although inhibitors of *P*-gp and breast cancer resistance protein have been used effectively to overcome the drug resistance in experimental animals, human data are still lacking.[52–54]

## Basement membrane

The basement membrane of the capillary wall in the brain is structurally an organized protein sheet with a thickness of 50–100 nm and surrounds both endothelial cells and pericytes. It is a highly dynamic constituent of the BBB and plays an essential role in the maintenance of BBB integrity.[55–57] The contents of the basement membrane are secreted by endothelial cells and pericytes and are mainly composed of laminins, collagen type IV isoforms, fibrillins, vitronectin, fibronectin, elastin, nidogens, and heparan sulfate.[56,58] In addition, soluble factors (e.g., growth factors and cytokines),

enzymes responsible for matrix degradation, and proteins such as lectins and semaphorins are also present in the basement membrane structure.[56,59,60] Laminins primarily play a role in the organization and scaffolding, and collagen type IV is essential for the stability of the basement membrane.[59,61] Matrix metalloproteinases that are activated by certain pathological insults may disrupt the integrity of the basement membrane resulting in BBB breakdown through an impairment in the functional activity of TJ proteins.[19,22]

## Pericytes

Pericytes, one of the components of the neurovascular unit, are located between endothelial cells and astrocyte endfeet (Figure 1). They are embedded in the same basement membrane surrounding the endothelial cells and thus are physically separated from both endothelial cells and astrocyte endfeet.[3,62,63] The available data on the percentage of the surface area of the abluminal plasma membrane of endothelial cells covered by the pericytes are contradictory with reported values ranging from 22 to 99% (Figure 3).[64–68] Pericytes are contractile cells, which provide physical support to and determine the vasodynamic properties of brain capillaries and hence contribute to the regulation of cerebral blood flow by controlling the luminal diameter.[69–71] A recent study suggested that pericytes can construct tunneling nanotubes that regulate neurovascular coupling and control capillary blood flow.[72] Pericytes are also essential for the induction of barrier characteristics in the endothelial cells including the formation of TJs and are involved in the regulation of BBB integrity and the transport of substances into the brain parenchyma.[62,63,73–76] Moreover, pericytes produce extracellular matrix proteins and play a crucial role in the regulation of endothelial cell proliferation, migration, and differentiation.[77–80] Besides, they also take part in the clearance of toxic cellular byproducts.[66,73,74,81] In experimental animals, pericyte deficiency induced by a platelet-derived growth factor mutation has been shown to cause a reduction in the expression of certain TJ proteins leading to a substantial BBB disruption.[63,73]

## Astrocytes

Astrocytes, the most abundant cell type in the brain, are characterized by their expression of the intermediate filament glial fibrillary acidic protein (GFAP) and their numerous cellular processes extending from the cell body. Most of these processes terminate as endfeet, which contact with the abluminal side of the basement membrane of brain capillaries to interact with endothelial cells and pericytes. Astrocytic perivascular endfeet are estimated to cover over 99% of the brain microvasculature wall (Figures 1 and 4).[23,42,65]

As a component of the neurovascular unit, astrocytes contribute to the regulation of vascular tone and local blood flow into the brain parenchyma and hence play an important role in the transport of oxygen and nutrients to neurons to maintain brain homeostasis. Astrocytes are the main actors that determine neuronal activity by regulating ion concentration and extracellular pH within the interstitial space and the uptake of glutamate and GABA in the synaptic region.[82–84] They express aquaporin-4 water channel proteins that promote perivascular clearance of waste material and hence form the newly characterized “glymphatic system” (CNS waste clearance system). Astrocytes also express certain transporters such as *P*-gp and Glut-1 along with Kir4.1 K+ channel proteins that aid in the maintenance of the neuronal resting membrane potential by removing extracellular K +.[85–89]

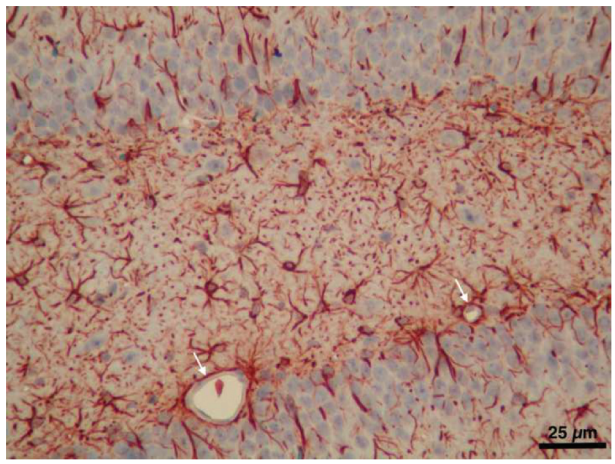


**Figure 4.** A light micrograph from our image archive showing astrocytes labeled by immunostaining for GFAP in the hippocampal region of the brain. Note the microvessels almost entirely surrounded with astrocyte endfeet (arrows) .

Astrocytes orchestrate the development of BBB properties and barrier maturation by releasing specific factors .[19,42,90–92] They are involved in the maintenance of BBB integrity by providing functional and anatomical support.[93,94] The alterations in astrocyte characteristics are reported to be associated with impairment in BBB integrity.[95] Data from the studies of our research group have shown BBB disruption along with alteration in GFAP immunoreactivity in astrocytes in experimental models of hypertension, febrile seizures, and irradiation.[96–98] On the contrary, there are some reports in the literature that oppose the necessity of glial cells for the maintenance of BBB integrity.[99,100] Furthermore, reactive astrocytes were found to disrupt the BBB integrity by releasing vascular endothelial growth factor.[101]

## Microglia

Microglia are long-living resident immune cells of the CNS and account for around 12–16% of the total cell population in the brain. They constitute the major cell type that acts in the protection of the brain against immunologic insults and thus contribute to the maintenance of neuronal homeostasis.[102,103] The breakdown of BBB may alter microglial activity through interaction with activated endothelial cells even in the absence of neurodegeneration, and hence brain regions with hypertrophied/activated microglial-like cells associated with vasculature may potentially display vascular damage and BBB compromise.[104] The activated microglia produce pro-inflammatory cytokines, including IL-1β and TNF-α, that further enhance the degree of BBB disruption.[105,106] Activation of microglia by lipopolysaccharide has been shown to decrease transendothelial electrical resistance (TEER) by disrupting TJ proteins, including claudin-5 and zonula occludens (ZO)-1 in an in vitro model of BBB.[107] Although microglia are defined as the cells that form the first line of defense against immunologic compromise, a putative role of these cells with regard to the maintenance of BBB integrity in physiologic conditions or restoration of disrupted BBB in the course of CNS diseases/disorders remains unclear.

## Neuron

Almost every neuron in the human brain is estimated to be nourished by a capillary microvessel positioned within an average distance of 15 μm.[108,109] Accumulated data have demonstrated that there is a direct interaction between neurons and barrier type endothelial cells, pericytes, as well as astrocytes.[22,23,110,111] The soma, axon, or dendrite of a neuron in close proximity to a brain capillary may contact pericytes and endothelial cells via the basement membrane.[6,112] Communication of neurons with glial and endothelial cells is essential for their survival and functions. Depending on metabolic requirements, neurons not only modulate various endothelial cell functions, including permeability, by activating specific enzymes expressed by the endothelial cells but also release growth factors to stimulate angiogenesis.[19,113,114] In the meantime, alterations in neuronal activity have been reported to affect BBB integrity.[115] Trans-endothelial electrical resistance and expression of TJ proteins have been found to be positively influenced when endothelial cells were co-cultured with neurons in vitro.[116]

## Functions of the blood-brain barrier

Brain capillaries, like their peripheral counterparts, essentially provide oxygen and nutrients to parenchymal cells, including neurons, and remove the produced waste materials. Importantly, endothelial cells of the microvessels in the brain display strong barrier properties which enable strict control of ionic and fluid movements between the circulation and the brain parenchyma to regulate the neuronal microenvironment. The transfer of substances from the blood to the brain parenchyma across the BBB is accomplished through a transcellular route in which receptor and carrier-mediated transporters are critical for transcytosis, and paracellular route in which TJs are the chief determinants of permeability.

## Characteristics of the blood-brain barrier

The endothelial cells of the brain capillaries are the fundamental anatomical structures of the BBB. These cells express not only a variety of specific

transport and carrier proteins which enable tightly controlled trafficking of molecules, but also TJ proteins which account for one of the barrier characteristics reflected by high electrical resistance (approximately 1800 Ω $cm^2$) compared with that in peripheral capillaries (2–20 ohm $cm^2$).[117,118]

### *Tight junctions*

The intercellular cleft between adjacent endothelial cells of brain capillaries houses two major types of junctional complex; TJs and adherence junctions (Figure 5Figure 6Figure 7). Tight junctions are highly dynamic structures that effectively limit the movement of water and solutes and regulate lateral diffusion through the paracellular pathway.[22,23,119,120] These structures are formed by transmembrane proteins claudins, occludin, and junctional adhesion molecules (JAMs) which interact with the actin cytoskeleton of the endothelial cells by a number of cytoplasmic accessory proteins including ZO proteins, cingulin, AF-6, and 7H6.[22,121–124] The interaction between ZO proteins and transmembrane proteins, including claudins and occludin have been shown to determine the

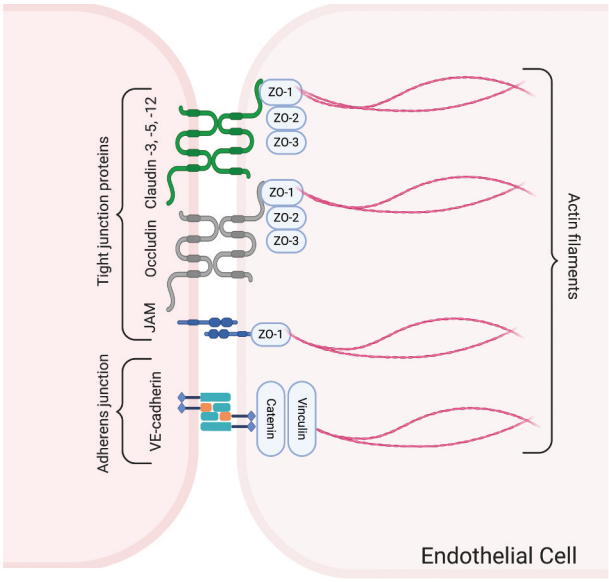


**Figure 5.** Schematic drawing of the junctional complex between barrier type endothelial cells of the brain, which controls the trafficking of substances through the paracellular pathway. The major proteins comprising TJs and adherens junctions and their linkage to the actin cytoskeleton are illustrated. Created with BioRender.com.

stability and function of TJs.[22,125–127] In contrast to the epithelial cells which exhibit intercellular gaps sealed by TJs localized at the most apical point of cellular attachment immediately above the clearly distinguishable adherens junctions, barrier type endothelial cells in the brain are joined together by TJs and adherens junctions showing more variable localizations and intermingled appearances.[128]

There are currently defined 27 members of claudins, and a number of them including claudin-1, −3, −5, −11, and −12 have been identified in the TJs between barrier type endothelial cells.[19,129–132] However, only claudin-1, −3, and −5 are most likely responsible for controlling the paracellular pathway while the roles of claudin-11 and 12 with regard to barrier function are yet to be elucidated.[23,130,133–135] Claudin-5 is the most enriched isoform in the brain endothelium and determines the sealing properties of TJs of the BBB.[134] Claudin-5 knockout mice display a selective increase in paracellular permeability for small molecules.[130] On the other hand, it is suggested that claudin-12 is not directly involved in the establishment or maintenance of BBB integrity.[136]

Occludin, the first TJ transmembrane protein described, has been defined as one of the major TJ proteins that controls the paracellular pathway of BBB.[125,137] Early studies showed an enhancement in TEER and reduction in paracellular diffusion by an increase in the expression of the occludin protein.[138,139] Moreover, dephosphorylation of occludin caused BBB failure in an experimental model of multiple sclerosis.[140] An autopsy series of fatal human septic cases showed that occludin expression in the barrier type endothelial cells was lost in the brain.[141] In diabetic rats, occludin and ZO-1 expression were decreased concomitant with an increase in BBB permeability to 14 C-sucrose.[142] Data from the studies of our research group have shown BBB disruption along with a reduction of occludin immunoreactivity in barrier type endothelial cells in in vivo experimental models of sepsis, hypertension, and irradiation.[97,98,143,144] In contrast, it is also suggested that occludin does not have the ability to establish TJ structure by itself but rather exerts a regulatory function on the barrier properties.[145] Accordingly, occludin-deficient mice display well-developed TJ complexes without any evidence of BBB hyperpermeability.[139]

Among the three isoforms of JAMs, JAM-1, JAM-2, and JAM-3, an interaction with occludin and claudins in TJs of brain capillaries to provide cell-to-cell adhesion has been described for JAM-1.[123,133,146] The members of JAM family also play an important role in leukocyte adhesion and transmigration to the brain parenchyma across BBB.[147]

Zonula occludens proteins (ZO-1, ZO-2, and ZO-3) are membrane-associated guanylate kinase homologs located in the cytoplasmic domain of TJs between endothelial cells. Among the members of ZO protein family, ZO-1 plays a central role in the assembly and organization of claudins, occludin, and JAMs, and links these TJ proteins to the cortical actin cytoskeleton.[133,148–150] ZO-1 is also a central regulator of vascular endothelial-cadherin–dependent adherens junctions that orchestrate the tuning of cell-cell tension, migration, angiogenesis, and barrier formation.[150] Data from our lab demonstrated decreased immunoreactivity for ZO-1 in brain capillary endothelial cells along with increased BBB permeability in an in vivo model of seizure and by radiation therapy in rats.[97,151] Besides, the loss or dissociation of ZO-1 and occludin from the junctional complex associated with BBB disruption have been shown in a variety of pathological conditions such as hypoxia, subarachnoidal hemorrhage, and Parkinson's disease.[97,151–154]

### *Adherens junctions*

Adherens junctions are constituted by $Ca^{1+}$-dependent transmembrane cadherin proteins that form homotypic adhesive complexes between neighboring endothelial cells and bind to the actin cytoskeleton via cytoplasmic anchoring proteins called catenins.[3,155,156] Although the primary function of adherens junctions is the attachment of adjacent endothelial cells, they may also involve in the formation and maintenance of tightness of the TJs.[119] However, the role of these junctional complexes in the development and normal physiology of BBB remains to be elucidated. Certain pathologic conditions causing disruption of adherens junctional proteins are associated with a loss of BBB integrity.[123]

## Transport pathways across the blood-brain barrier

Under physiological conditions, the BBB exhibits low permeability compared to peripheral blood vessels.[157] Lipid solubility, electrical charge, molecular size, and hydrogen bonding capacity are the main determinants of the ability of a molecule in circulation to enter the brain across an intact functioning BBB. The paracellular passage of molecules is considerably minimized, while ions and solutes can diffuse between adjacent cells according to their concentration gradients. On the other hand, most of the nonpolar lipid-soluble molecules of small molecular weight (<400-500 Da) such as carbon dioxide, nitric oxide, ethanol, and oxygen, are readily transported into the brain parenchyma by the process of the passive diffusion mainly via transcellular route through the lipid bilayer of the endothelial cell membrane.[3,12,16,42,158,159]

Hydrophilic and charged molecules can only penetrate the BBB by active transport systems, including receptor-mediated transport, carrier-mediated transport, and adsorptive-mediated transport to enter the brain.[42] Molecules such as glucose, transferrin, and amino acids and ions, including potassium, sodium, calcium, and bicarbonate, utilize these active transport systems; however, growth factors and cytokines have limited ability to permeate across the BBB.[160,161] On the other hand, efflux transporters such as *P*-gp pump their substrates such as drugs and metabolites back to the circulation.[162–164]

## The paracellular pathway

The discovery of the ultrastructure of BBB enabled a clear understanding of the trafficking of substances across the barrier, and the paracellular pathway strictly controlled and regulated by the TJs localized along the interendothelial space was described.[8] The specialized TJ proteins effectively prevent undesirable passive diffusion of lipophilic or low molecular weight substances and passage of immune cells through the gaps between the endothelial cells of brain capillaries and provide an obstacle for the bulk flow of water and plasma-sourced solutes by the paracellular route.

## The transendothelial pathway

The trafficking of substances across the barrier type brain capillary endothelial cells is primarily mediated by the transcellular route. As a prominent characteristic of the barrier type endothelial cells, this pathway utilizes various types of influx transporters collectively called nutrient transporters, while efflux transporters pump their specific substrates back to the bloodstream (Figure 6).[44,165,166] The transport of molecules by the transcellular pathway is bidirectional using receptor and carrier proteins located on both luminal and abluminal membranes of barrier type endothelial cells by energy-dependent or -independent processes.[167] In addition, fluid-phase and adsorptive endocytosis is used to transport some non-lipid-soluble molecules of small molecular weight and macromolecules like albumin, immunoglobulins, and other proteins.[168,169]

### *Receptor-mediated transport*

The main pathway in the trafficking of molecules across the BBB is receptor-mediated transcytosis which mediates the transport of the circulatory substances including transferrin, low-density lipoproteins, leptin, insulin, and insulin-like growth factor into the brain; however, a shift to ligand-nonspecific caveolar transcytosis is observed by aging.[33,41,170–173] Receptor-mediated transcytosis has recently been the focus of interest in targeted drug delivery studies in which molecular Trojan horses, vectors that can bind specific receptors of the pathway, are used to enable the transport of drugs into the brain at effective doses in certain CNS diseases/disorder resistant to pharmacological treatment.[174,175]

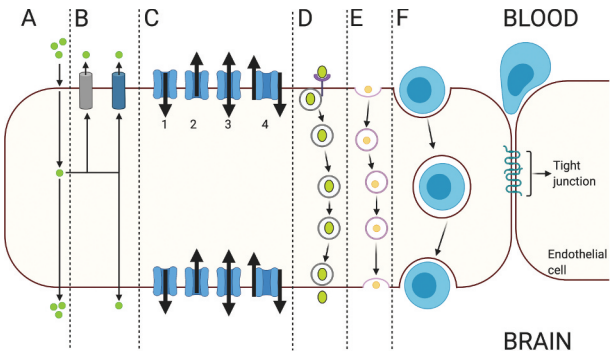


**Figure 6.** The schematic drawing of various routes of transcellular transport across barrier type endothelial cells in the brain. A: passive diffusion, B: efflux transport, C: carrier-mediated transport, D: receptor-mediated transport, E: adsorptive-mediated transport, F; cellular transport. Created with BioRender.com.

### *Carrier-mediated transport*

The circulatory-sourced vital substances that are essential for the energy and neurotransmitter metabolism in the brain, including nutrients such as glucose, vitamins, and hormones require the carrier-mediated transporters located both on luminal and abluminal plasma membranes of barrier type of endothelial cells to reach into the brain parenchyma through a saturable transport process.[2,19,31,176–180] Glut-1 mediates the uptake of D-glucose, the main energy source of the brain, by barrier type endothelial cells and delivery to astrocytes and neurons.[181–183] In the opposite direction, the uptake of D-glucose from the brain interstitium into the circulation is accomplished by the Na+-D-glucose cotransporter Sglt1, expressed in the brain capillary endothelial cells, which further contributes to the adjustment of glucose concentration in the brain interstitium.[183] Glut-1 is also reported to be crucial for angiogenesis during brain development.[184] The transport of certain ions in exchange of or simultaneously with other ions is exerted by exchanger pumps including sodium/potassium pump and sodium-hydrogen, chloride-bicarbonate and sodium-calcium exchangers, and cotransporters such as sodium-potassium-two chloride cotransporter localized on the abluminal and/or luminal side of the barrier type endothelial cells.[185–187]

### *Caveolae-mediated endocytosis*

Caveolae are characteristic flask-shaped membrane invaginations with a diameter of 50–80 nm, which are mainly responsible for endothelial transcytosis in barrier type of brain capillary endothelial cells.[188,189] The caveolar membranes contain caveolin-1/2 and vesicle-associated membrane protein-2 as well as receptors for certain essential enzymes, hormones, plasma carrier proteins, and cytokines.[190,191] Caveolin 1, which is the principal component of caveolae, can also influence the expression of TJ proteins.[192] The expression of the caveolin-1 is significantly increased in barrier type endothelial cells under several pathological

conditions and by aging.[193–195] Major Facilitator Superfamily Domain containing 2a (Mfsd2a), a lipid transporter highly expressed in the endothelial cells of brain microvessels, inhibits caveolae production and hence plays an important role in barrier characteristics.[196–198] Therefore, brain capillary endothelial cells exhibit few caveolae, whereas arteriolar endothelial cells in which Mfsd2a transcript levels are low display abundant caveolae.[199,200] Knock-out of Mfsd2a in mice caused increased caveolae production and transcellular permeability in the brain microvasculature.[195,197]

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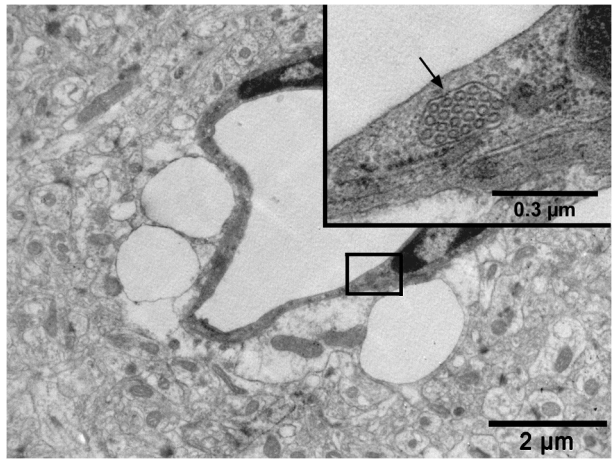


**Figure 7.** An electron micrograph from a previous study from our research group (reproduced from Ref. # 96 with permission from Elsevier Science) showing a capillary from the hippocampus region of the brain of a rat with cortical dysplasia exposed to febrile seizures. Note a conglomerate of caveolar vesicles within a cargo (arrow in the inset) in the cytoplasm of a brain capillary endothelial cell and the intensive pericapillary edema with swollen astrocyte endfeet.

### *Efflux transport*

The multidrug-resistance proteins primarily consist of multidrug-resistance protein-1 or *P*-gp, multidrug resistance-associated protein, and breast cancer resistance protein.[202] The *P*-gp, as an efflux pump, is located on the luminal side of the BBB and restricts the permeation of a large number of toxins into the brain parenchyma, providing neuroprotection and detoxification.[203–205] On the other hand, the efflux pumps also restrict the entry of therapeutic agents, including antibiotics, chemotherapeutics, and antiepileptic drugs into the brain parenchyma at effective doses and thus severely contribute to the development of pharmacoresistance in the treatment of certain types of brain tumors and epilepsy.[44,202,206]

## Cell movement across the BBB

Under inflammatory conditions, circulatory-sourced immune cells and neutrophils can penetrate into the brain via paracellular pathway which requires opening and rearrangement of TJ complexes and transendothelial pathways which involves the dynamic organization of cellular processes of leukocytes called invadosomes and various vesicles and vesiculovacuolar organelles forming a transcellular pore through the endothelial cells.[207–210] The cell surface levels of endothelial intercellular adhesion molecule-1 and caveolin-1 also play a crucial role in transcellular immune cell entry into the brain.[211,212]

## Circumventricular organs

In contrast to the capillaries located in the brain parenchyma, blood microvessels in the circumventricular organs do not display barrier properties. The endothelial cells of these microvessels have fenestrae, which allow the free diffusion of substances between the blood and CNS.[37,213,214] These organs consist of secretory structures like pineal gland, subcommisural organ, median eminence, and choroid plexuses and sensory regions, including area postrema, subfornical organ, and organum vasculosum of the lamina terminalis.[215] The exchange of hormones and other molecules between the circulation and CNS is accomplished mainly in circumventricular organs in which increased vascularization facilitates the sensory and secretory roles to mediate the communication between the brain and the periphery.[215,216]

## BBB disruption in pathological conditions

While the integrity of the BBB is crucial for the maintenance of neuronal homeostasis, alterations in functional and structural properties of the barrier are closely interrelated with the occurrence of certain brain pathologies. A variety of CNS diseases/disorders including epilepsy,[175,217] ischemic stroke,[218] multiple sclerosis,[219] traumatic brain injury,[220] and Alzheimer's disease[221] are characterized by BBB disruption. On the other hand, CNS manifestations associated with BBB breakdown may develop in systemic diseases such as sepsis[222,223] and hypertension.[201]

## Drug delivery into the brain for clinic implications

Innovative strategies have been developed for overcoming BBB to enable the access of therapeutic drugs at effective doses in CNS diseases/disorders in experimental settings; however, their current use in clinical practice is still limited. Circumventing BBB by temporarily disrupting the TJs between brain capillary endothelial cells has been reported to allow the access of pharmacologic agents into the CNS in both experimental animals and humans. The intravenous administration of bradykinin and histamine, intraarterial infusion of hyperosmolar solutions like mannitol and application of transcranial focused ultrasound together with microbubbles temporarily open the TJs between barrier type endothelial cells in the brain.[224–226] Moreover, conjugating the pharmacological agents with nanocarriers such as liposomes, nanopolymers, nanoparticles, viruses, and exosomes which normally have access into the brain using the endothelial transcellular transport mechanisms is being extensively studied in the last decade as an alternative approach to the drug delivery into the brain[43,227–229]

## Future challenges

Our understanding of the transport dynamics across BBB in both physiological and pathological conditions has advanced considerably in recent years by the rapid development of advanced molecular techniques, imaging modalities, and nanotechnology. In this context, the accumulated data on the behavior of BBB will pave the way to elucidate the mechanisms underlying the response of the neurovascular unit in neurodegenerative disorders/diseases and to develop novel therapeutic strategies. On the other hand, exploiting ways for the targeted delivery of pharmacologically active substances into the brain through transendothelial transport pathways using nanocarriers or by the reversible opening of both transcellular and paracellular routes will allow BBB permeation of therapeutic agents at effective doses. We believe that an elaborate network of expertise with the collaboration of researchers from various disciplines, including medicine, chemistry, bioengineering, and electronics may enable us to overcome future challenges.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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