HIGH SCHOOL EDITION
Journal of Student Research
Volume 10 Issue 4 (2021)

Exploring the Blood Brain Barrier: Structure, Function, and Medical Implications

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ABSTRACT

The blood brain barrier is the brain's physical defense against pathogens and substances that may alter cerebral homeostasis. It is formed under instruction of neurons via astrocytes, and primarily consists of tightly junctioned brain capillary endothelial cells and pericytes. In this review, we first discuss the structure and function of the blood brain barrier, including the variety of transport methods incorporated to maintain cerebral homeostasis, such as transport channels, membrane transport, carrier mediated transcytosis, receptor mediated transcytosis, adsorption mediated transcytosis, and efflux systems. Then, we focus on the three pathways of pathogenic invasion of the blood brain barrier- paracellular, transcellular, and the "Trojan Horse" mechanism, and elaborate specifically on the capability of the SI protein on the SARS-CoV-2 virus to cross the blood brain barrier, causing encephalitis. Finally, we explore the challenge the blood brain barrier's extremely low vascular permeability poses on medicine and attempts to overcome these challenges using tDCS and nanocarriers. Thus, the blood brain barrier, its structure and function, and its scientific and medical implications remain intriguing frontiers of science.

Introduction

The brain has the most airtight defense system out of all the organs in the human body, as an inflammation in the brain would possibly yield more severe and fatal outcomes compared to one in any other organ. One of these systems of defense unique to the brain is the blood brain barrier (BBB). The blood brain barrier describes a decrease in vascular permeability in brain capillaries that creates a physical barricade, preventing a variety of potentially harmful substances in blood from entering cerebral blood flow and thus brain parenchyma (Ballabh *et al.*, 2004). Its extremely low vascular permeability is also vital in maintaining homeostasis in the brain such that fluctuations in ions, hormones, and neuroactive substances in the body have less effect on the brain (Segarra *et al.*, 2021). The blood brain barrier achieves such low vascular permeability via increasing cell density and thickness of brain capillary walls, causing them to be less porous (Figure 1).

Endothelial Cells and Pericytes

Endothelial cells and pericytes are cells that form the walls of blood vessels. Endothelial cells line up next to each other and fold to create the lumen. Where endothelial cells meet is a cell junction formed by a junctional complex, proteins located in/on endothelial cell membranes that facilitate contact and/or adhesion between two endothelial cells. Brain capillary endothelial cells (BCECs) form a type of junctional complex called a tight junction, meaning that BCECs are bound to each other diagonally, forming a watertight seal (González-Mariscal *et al.*, 2003). In comparison, endothelial cells of peripheral capillaries are bound to each other side by side, forming a less watertight seal.

On top of the endothelial cells is a vascular basement membrane- a 3 dimensional protein matrix consisting of proteins such as laminin, collagen IV, nidogen, and heparan sulfate proteoglycans secreted by BCECs and pericytes

(Thomsen et al., 2017). Vascular basement membranes function to provide physical support and scaffolding required for epithelial tissue maintenance and regulate vascular permeability (Thomsen et al., 2017). Brain capillaries have a greater pericyte density in their vascular basement membranes compared to peripheral capillaries, causing brain capillaries to have thicker and denser vascular basement membranes, thus lowering brain capillary permeability.

Astrocytes

On top of a denser and thicker vascular basement membrane, brain capillaries have a unique second membrane, sometimes known as the glial basement membrane. This membrane is formed by astrocytes, a kind of glial cells, or cells that support the nervous system's functions (Jäkel, Dimou, 2017). Astrocytes secrete a protein matrix on top of the vascular basement membrane and are responsible for the formation and maintenance of the blood brain barrier as it secretes the factors required for BCECs and pericytes to assemble in the previously discussed manner (Cabezas *et al.*, 2014). Moreover, astrocytes modulate neuronal activity and cerebral blood flow and maintain homeostasis by connecting brain capillaries with neurons using their endfeet, the part of astrocytes that attach on the vascular basement membrane to form the glial basement membrane, allowing astrocytes to pass on neural signals from neurons to blood vessels in the brain (Daneman, Prat, 2015). Astrocyte endfeet also express proteins necessary for molecular transport between capillaries and brain tissue and take part in regulating ion levels in the blood brain barrier via Ca^{2+} polarization (Daneman, Prat, 2015).

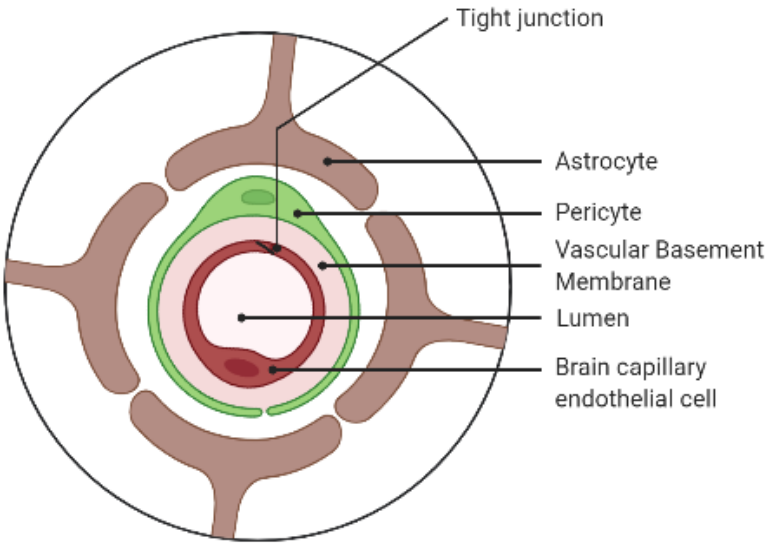

Figure 1. Cross Section of the Blood Brain Barrier

Transport through the Blood Brain Barrier

The key criteria that allows a molecule to cross the blood brain barrier is small size, though 98% of small molecules are unable to pass through. Numerous mechanisms (Figure 2) are incorporated by the blood brain barrier to transport assorted molecules from circulation to cerebral tissue. Small molecules, such as oxygen and carbon dioxide enter and exit cerebral tissue via passive diffusion following a concentration gradient. These molecules have to be hydrophilic such that they are soluble in water, but also lipophilic enough that they can pass through the phospholipid bilayer membrane of endothelial cells. Small ions, such as Na^+, K^+, and Cl^- and water are able to pass through specialized

protein channels in endothelial cell membranes. Small polar (hydrophilic) solutes, such as amino acids, glucose, organic anions and cations, and nucleosides are transferred via a carrier mediated transport system specific to one or few molecules. Larger polar solutes, like hormones, cross the blood brain barrier via receptor mediated endocytosis. Cytokines, a signaling molecule synthesized by immune cells, are selectively transported across the blood brain barrier by receptors. The difference between these two mechanisms is that carrier mediated transport utilizes intrinsic and extrinsic protein channels where the binding of the solute to a receptor causes conformational changes in the protein channel, to allow the solute's passage through the protein channel (Alberts et al., 2002), while receptor mediated transport essentially phagocytizes the solute, which is bound to a receptor on the cell membrane. Another way in which larger solutes cross the blood brain barrier is transcytosis, a process in which the solute is endocytosed by an BCEC, creating a vesicle which travels across the BCEC interior to be exocytosed into brain tissue (Hervé et al., 2008). Transcytosis in the blood brain barrier can be both receptor mediated and adsorption mediated. Receptor mediated transcytosis relies on the binding of a molecule or a part of it to receptor proteins on BCEC membranes specific to one or a few molecules. Adsorption mediated transcytosis utilizes non-specific molecules to BCEC membrane interactions resulting in the budding of a vesicle around the molecule.

In addition to a highly regulated, complex system of proteins responsible for acting as a filter for selective transport, the blood brain barrier has an efficient efflux system, which is essential in maintaining cerebral homeostasis. The efflux system, a protein complex that pumps out excess or toxic molecules, acts as a second layer of regulation for the blood brain barrier. Efflux proteins in the blood brain barrier are especially crucial in regulating passive diffusion.

The very limited amount of inorganic molecules that are able to cross the blood brain barrier are small non-polar molecules capable of diffusing into the brain at high concentrations by following a concentration gradient. Some examples of these molecules include alcohol, recreational drugs, and mental illness medication. However, most drugs are too big to diffuse into brain tissue, resulting in difficulties delivering them orally and having to be administered via intracranial injections, an invasive procedure. Therefore, investigations into enabling the crossing of medical drugs into the brain, whether by means of manipulating blood brain barrier permeability or creating molecules capable of crossing the blood brain barrier, remain an intriguing conundrum to researchers.

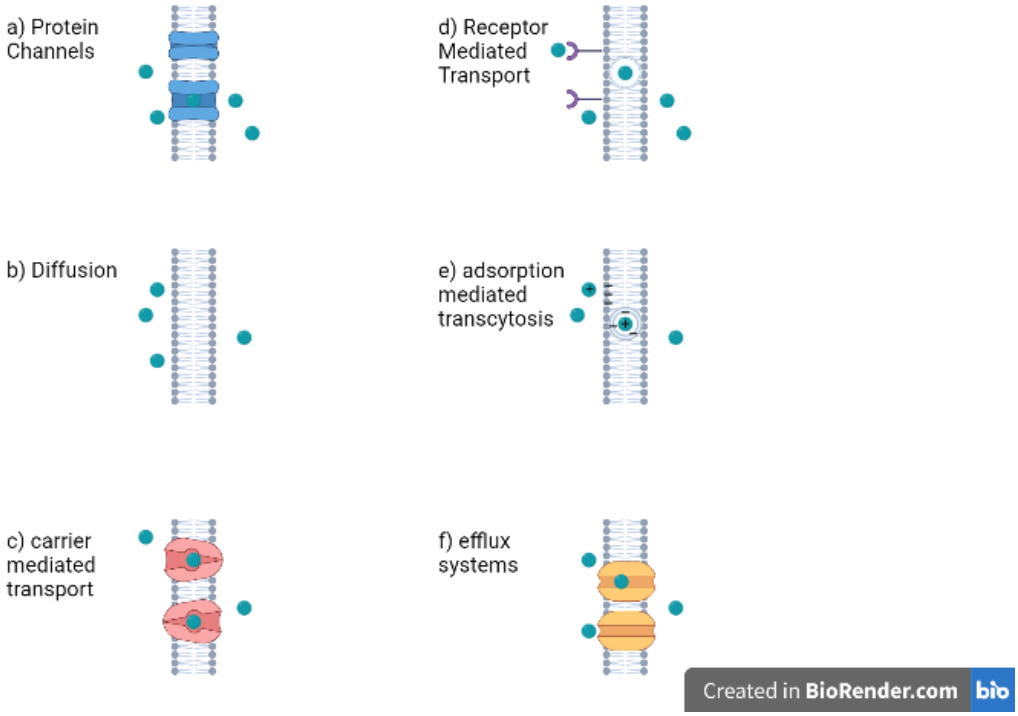

Figure 2. Transport Systems at the Blood Brain Barrier

Blood Brain Barrier Infection

Despite the blood brain barrier's extremely selective permeability, which creates an airtight defense, pathogens co-evolve systems to pass it. The three main ways (Figure 3) pathogens do so are transcellular entry, paracellular entry, and a method known as the Trojan Horse (Chen, Li, 2021). Pathogens capable of invasion via the transcellular pathway essentially utilize structures that enable transcytosis through a BCEC. Such pathogens may have a surface protein that enables it to undergo receptor mediated transcytosis by binding to BCEC receptor proteins, such as transferrin receptor, insulin receptor and low density lipoprotein receptor-related proteins 1 and 2 (LRP1 and 2) (ASM, 2020). Examples of bacterial surface proteins capable of such include IbeA, IbeB, AslA,YijP, OmpA, PilC and InlB (Huang, Jong, 2001). Other pathogens are able to invade cerebral tissue by facilitating charge reactions with BCEC membranes necessary to initiate adsorption mediated transcytosis. As these reactions are non specific, a larger variety of microbes are able to invade brain parenchyma using this method as opposed to receptor mediated transcytosis, which is highly specific. Pathogens that employ this method include *Escherichia coli*, group B *Streptococcus*, *Streptococcus pneumoniae*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, fungal pathogens such as *Candida albicans*, and *Cryptococcus neoformans*, and is suggested for the West Nile virus (WNV) (Pulzova *et al.*, 2009).

Pathogens that take the paracellular pathway pass through the blood brain barrier by disrupting the tight junctions between BCECs (ASM, 2020). Pathogenic attachment to BCEC junctions are a sign of paracellular infection. Fewer pathogens employ this method of invasion compared to the transcellular pathway, as in principle, this method is more complex (ASM, 2020). Pathogens may disrupt tight junctions by expressing proteins that loosen or break them apart. Some pathogens do so by secreting apoptotic factors that cause apoptosis in BCECs, enabling entry (Pulzova *et al.*, 2009). Another way for paracellular entry of pathogens is to initiate a "false alarm" immune response that causes cytokines or chemokines to breach the tight junctions between BCECs to enter the brain, thus increasing brain vascular

permeability and allowing the pathogen and/or other pathogens to enter brain parenchyma. The *Trypanosoma sp.* are an example of pathogens that invade through the paracellular path (Yeager, 2019).

The third pathway of invasion is known as the "Trojan Horse" mechanism. Pathogens using this method of invasion infect a type of leukocytes called phagocytes, which the blood brain barrier is permeable to (ASM, 2019). Phagocytes and some other immune system cells and substances such as cytokines regularly enter cerebral circulation and brain parenchyma as they circulate throughout the body, surveilling for foreign material and phagocytizing them. Pathogens become passengers inside a host phagocyte (Santiago *et al.*, 2017). Such pathogens include *L. monocytogenes, Mycobacterium tuberculosis,* and HIV (Pulzova *et al.*, 2009).

Various health conditions may increase vulnerability to cerebral infections as they cause a decrease in blood brain barrier permeability. For instance, neurodegenerative diseases cause progressive loss and function of the nervous system. As the blood brain barrier is formed under the instruction of and regulated by neurons, neurodegenerative diseases decrease the nervous system's ability to form an airtight blood brain barrier, resulting in increased permeability and thus risk of infection. For the same reason, cerebral injuries also increase blood brain barrier permeability. Certain autoimmune diseases that affect the brain, such as autoimmune encephalitis, in which the immune system attacks healthy cells and tissue in the central nervous system, also cause blood brain barrier permeability to decrease.

In light of the ongoing Covid-19 pandemic, it is crucial to note that the S1 spike protein on the SARS-CoV-2 virus has been shown to be capable of crossing the blood brain barrier via adsorptive transcytosis in male mice. Encephalitis has been known to occur in Covid-19 patients, and SARS-CoV-2 viral mRNA has been recovered from cerebrospinal fluid, demonstrating that SARS-CoV-2 is capable of crossing the blood brain barrier. It has also been proven that the 2003 SARS coronavirus, another strain of human coronavirus (HCoVs), is able to cross the blood brain barrier. Brain parenchymal infection caused by Covid-19 is a possible cause of nervous system related symptoms including loss of taste and smell, headaches, twitching, seizures, confusion, vision impairment, nerve pain, dizziness, impaired consciousness, nausea and vomiting, hemiplegia, ataxia, stroke, and cerebral hemorrhage. However, nervous system related symptoms may also be caused by cytokine storms, which are induced by SARS-CoV-2 infection, where the body secretes immense amounts of cytokines to combat the infection, causing cytokine levels in the brain to rise and affect nervous system function. It has also been suggested that some Covid-19 symptoms may be caused by viral infection of the central nervous system. For example, respiratory symptoms may be a result of infection of the respiratory center of the brain. (Rhea *et al.*, 2021)

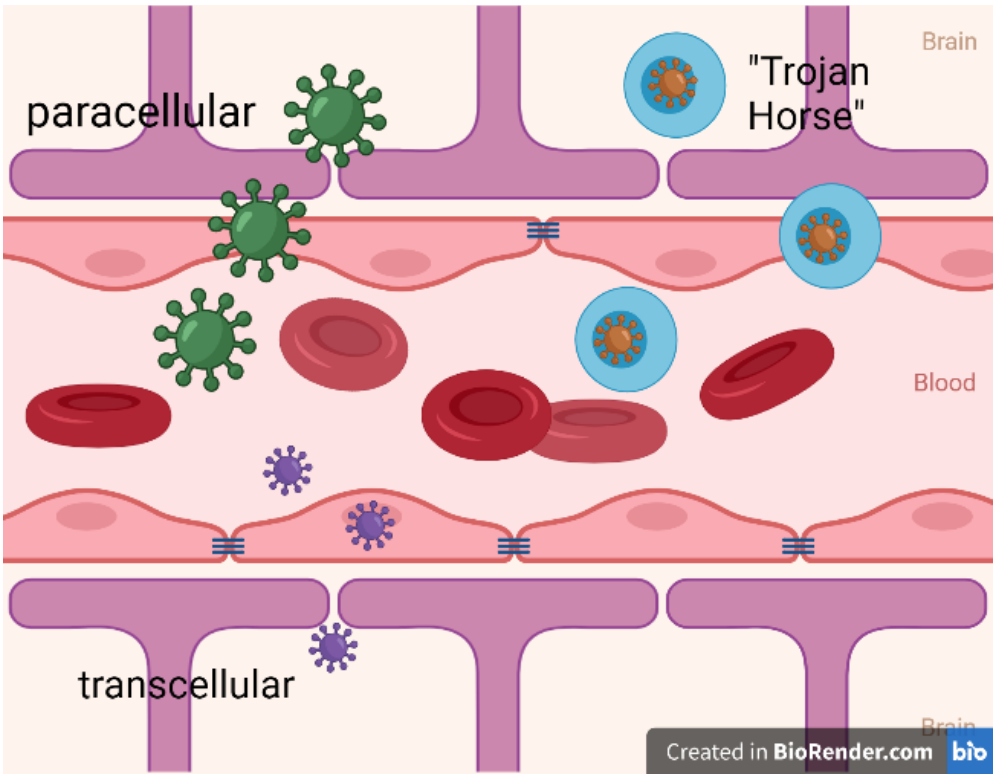

Figure 3. Pathways of Blood Brain Barrier Invasion

Implications of the Blood Brain Barrier in Future Medicine

The blood brain barrier poses an obstacle in drug delivery to the brain such that its extremely low permeability prevents most medication, which are large, complex molecules, from entering the brain. As a result, numerous drugs targeting central nervous system diseases and neuroimaging radiopharmaceuticals have to be administered via intracranial injections and other invasive, unsustainable methods. This paper highlights two of many attempts to overcome this challenge.

Transcranial Direct Cranial Stimulation (tDCS) is a procedure developed with the goal of treating both mental illnesses and traumatic brain injuries (Center for BrainHealth, 2018) in which low amplitude (1.0-2.5mA) direct currents are passed through two electrodes placed on the head to excite or inhibit neuronal activity (Mennitto, 2019). As the blood brain barrier is formed by instruction of the brain, tDCS has been shown to be able to temporarily increase blood brain barrier permeability via partially inhibiting these instructions, allowing drug delivery from the bloodstream to brain parenchyma. It is also possible to model blood brain barrier permeability as a result of anode stimulation by tDCS, enabling physicians to adjust blood brain permeability based on the size of the drug used (Shin *et al.*, 2020). Nevertheless, adjustment of blood brain barrier permeability via tDCS, though a noninvasive procedure, is a rather cumbersome and complex procedure for drug delivery to the brain. tDCS is still considered an experimental procedure and is not FDA approved yet (Mennitto, 2019).

Nanocarriers have also been used to allow drug delivery across the blood brain barrier (Mulvihill *et al.*, 2020). Nanocarriers are molecules under 500 nm in diameter that act as transport modules for other substances, commonly medical drugs. Inorganic solid nanoparticles (NPs), lipid-based liposomes, solid-lipid NPs, polymer-based NPs and protein-based NPs are most commonly used as nanocarriers for drugs to cross the blood brain barrier (Din *et al.*, 2017). Nanocarriers employ a variety of methods, including receptor mediated transport and transcytosis, to cross the blood brain barrier, mostly having to do with their physical properties (Mulvihill *et al.*, 2020).

The blood brain barrier is a fascinating topic of study for both the academic and medical worlds due to its complexity and its crucial roles in organ systems of the body, such as the nervous, immune, and circulatory systems. Rapid progress in recent blood brain barrier research has led to increased understanding of the blood brain barrier's function and mechanisms. Thus, there are immense medical implications in understanding the blood brain barrier.

Acknowledgments

I would like to thank my mentor Janice Huang for helping me with this research.

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