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Anatomy and Physiology of the Blood-Brain Barrier

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Abstract:

Essential requisite for the preservation of normal brain activity is to maintain a narrow and stable homeostatic control in the neuronal environment of the CNS. Blood flow alterations and altered vessel permeability are considered key determinants in the pathophysiology of brain injuries. We will review the present-day literature on the anatomy, development and physiological mechanisms of the blood-brain barrier, a distinctive and tightly regulated interface between the CNS and the peripheral circulation, playing a crucial role in the maintenance of the strict environment required for normal brain function.

Keywords: blood-brain barrier; neurovascular unit; neuroglia; vessel permeability

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1. Anatomy of the blood-brain barrier

To maintain normal brain function, the neural environment must be preserved within a narrow homeostatic range; this requires a tight regulation of transportation of cells, molecules and ions between the blood and the brain. Such tight regulation is maintained by a unique anatomical and physiological barrier, formed collectively in the central nervous system (CNS). The existence of a physical interface between the CNS and the peripheral circulation and the vascular capacity was first described by Paul Ehrlich [1]. Ehrlich described how dye injection into the blood circulation stained peripheral organs but not the spinal cord and the brain**.** Later, Ehrlich's student Edwin Goldmann, showed that direct injection of trypan blue into the cerebrospinal fluid (CSF) stained cells within the CNS and not in the periphery [2]**.** Additional limiting element was later demonstrated by Reese and Karnovsky who presented a solute exchange barrier between the blood and the brain by means of an endothelial tight junction complexes [3].

Three barrier layers contribute to the separation of the blood and neural tissues: (1) a highly specialized endothelial cells (EC) layer comprising the blood-brain barrier (BBB) and partitioning the blood and brain interstitial fluid, (2) The blood-CSF barrier (BCSFB) with the choroid plexus epithelium which secretes the specialized cerebral spinal fluid (CSF) into the cerebral ventricles, and (3) the arachnoid epithelium separating the blood from the subarachnoid CSF [4].

The BBB components include the EC layer and its basement membrane, adjoined by tight cell-to-cell junction proteins with specific transport mechanisms and pinocytic vesicles. The endothelium is surrounded by cellular elements including pericytes and astroglial foot processes, forming an additional continuous stratum that separates blood vessels from brain tissue. Around penetrating vessels and venules there is some distance between EC and brain tissue forming the Virchow-Robin space in which perivascular macrophages, executing some of the immune functions of the CNS, are found. The intimate contact between neurons, astrocytes, microglia, pericytes and blood vessels, and the functional interactions and signaling between them form a dynamic functional unit, known as the ***neurovascular unit***. Understanding the function of the neurovascular unit is an important key to the understanding of brain functions in health

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and disease, including neuronal firing, synaptic plasticity, regulation of blood flow and response to injury (thoroughly reviewed by: [5])

The innermost luminal constituent of the neurovascular unit is comprised of a single specialized EC layer lining brain capillaries, exhibiting a greater number and volume of mitochondria, augmenting the selective molecular permeability of the BBB [6]. The basement membrane, a 30 to 40-nm thick lamina composed of collagen type IV, heparin sulfate proteoglycans, laminin, fibronectin, and other extracellular matrix proteins, encompasses pericytes and endothelial cells and is closely adjacent to the plasma membranes of astrocyte end-feet, enclosing the cerebral capillaries [7]. Transmembrane proteins (junctional adhesion molecule-1, occludin, and claudins1/3, 5, and possibly 12) and cytoplasmic accessory proteins (zonula occludens-1 and -2, cingulin, AF-6, and 7H6) establish the tight junctions between adjacent endothelial cells. Molecular and structural studies of tight junctions reveal a complex, dynamic and highly regulated molecular structure. Junctional adhesion molecules maintain tight junction properties, claudins facilitate tight barrier capabilities, occludins and zonula occludens-1 regulate targeted signaling [8–10].

Pericytes are enveloping brain microvessels and capillaries and are found in close proximity to astrocytes and neurons. The ratio of pericytes to endothelial cells is assessed to be 1:3 [11]. Using multiple signaling pathways, pericytes seems to play a critical role in the formation and maturation of the BBB during development and regulation of tissue-survival [12]. In addition, pericytes control over cerebral blood flow due to regulation of capillary diameter through actin fibers in the pericytic cell body [13]**.** Dysfunction of pericytes through aging was reported in animal models [14] and their absence results in loss of BBB integrity and reduction in regional cerebral blood flow [15].

Astrocytes interact with pericytes and microvascular endothelial cells by endfeet protrusions ensheathing the capillaries. Interactions may also exist with smooth muscle cells at arterioles. Astrocytes play important roles in maintenance of the BBB, in homeostasis of extracellular concentration of transmitters, metabolites, ions and water,

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but also serve as stem cells during development and provide for templates for migratory neuronal streams. Interaction between astrocytes and neurons determine synaptic transmission, clearance of neurotransmitters, plasticity, and blood flow (reviewed by [16]).

2. Development of the blood-brain barrier

A key developmental phase of the BBB lies in the early communications of the embryonic endothelium with neural cells [17]. The BBB matures during fetal life and is well formed by birth [18–23]. Transport mechanisms may continue to develop in mammals born in a relatively immature state (such as the rat and mouse) and become fully functional only in the peri- or post-natal period [24]. The development of the vascular endothelium is now known to be provoked by neuroepithelial signaling through Wnt/β-catenin pathway to induce a CNS-specific vascular system and BBB specialization [25–27].

An early feature of BBB development is the formation of tight junctions. In humans, a brain of a 14 week fetus express occludin and claudin-5 in the capillary endothelium with the same distribution at cell margins as seen in the adult [28]. Human post-mortem studies of perinatal deaths and stillborn fetuses from approximately 12 weeks gestation have demonstrated that a barrier to trypan blue exist from at least the beginning of the second trimester, equivalently to that of the adult human (Grontoft, 1954). Culture studies suggest that astrocytes have a key role in regulating the tightness of the BBB [30].

3. Physiology of the blood-brain barrier

Each of the three main CNS interface layers: the BBB, choroid plexus epithelium and the epithelium of the arachnoid mater, functions as a physical, transport, metabolic, and immunologic barrier. The barrier functions are dynamic and respond to regulatory signals from both blood and brain. Tight junctions between adjacent cells restrict

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diffusion of polar solutes through the intercellular cleft (paracellular pathway). The barriers are permeable to O_2 and CO_2 and other gaseous molecules such as helium, xenon, N_2 and many gaseous anesthetics. The permeability to xenon may provide a high resolution magnetic resonance imaging tool by which small morphological alteration may be detectable within the living tissue and also permit the analysis of binding sites using molecular probing techniques. Lipid soluble substances can pass the barrier by diffusion. Principally, the BBB is also permeable to water, however solute carriers on the apical and basal membranes together with ectoenzymes and endoenzymes regulate small solute entry and efflux. Transfer of some molecules is regulated by multidrug transporters that can limit their concentrations within the central nervous system. Multidrug transporters are ubiquitous transport proteins that exploit ATP hydrolysis to funnel molecules across lipid membranes; they facilitate transport of molecules into cells but may also prevent accumulation of molecules within the brain interstitial space. Multidrug transporters and Pgp-like proteins are expressed at the BBB and limit access of drugs to brain tissue but also other lipophilic molecules, including (for example) bilirubin, the degradation product of hemoglobin, which - if entering the central nervous system – is neurotoxic and can cause significant damage [31]. Recent studies suggest that upregulation of transporter molecules in pathological conditions may reduce drug levels within the brain, and explain treatment failures (pharmacoresistant) in neurological and psychiatric disorders [32].

Large molecules (e.g. peptides and proteins) with particular growth and signaling roles within the CNS enter the brain in a restricted and regulated manner by adsorptive and receptor-mediated transcytosis (ART and RMT, respectively). Smaller peptides may cross the BBB by either nonspecific fluid-phase endocytosis or RMT mechanisms. Similarly, 98% of all small molecules are not freely transported across the BBB [33]. The barriers also regulate the recruitment and entry of leukocytes and innate immune elements and involve in both the reactive and surveillance functions of CNS immunity. Leukocyte migration involves a complex set of adhesion molecules at the surface of leukocytes and vascular endothelial cells. Tethering and rolling of leukocytes is achieved via integrins VLA-4 (α4β1) and α4β7 [34] and adhesion molecules such as ICAM-1, VCAM-1 and PECAM-1, contribute to the adhesion and/or migration of distinct

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subsets of leukocytes to the CNS through cytokine-activated brain endothelium [35]. Transport systems across the blood-brain barrier are illustrated in Figure 1.

3.1 Transport of glucose and amino acids

Carrier-mediated influx, which may be passive or secondarily active, provide transport into the CNS of essential polar molecules that cannot diffuse through the cell membrane such as glucose, amino acids and nucleosides. Endothelial cells of the brain microvasculature, astrocytes, and the choroid plexus express the insulin independent glucose transporter GLUT1, a membrane-spanning glycoprotein containing 12 transmembrane domains with a single N-glycosylation site [36]. GLUT1 plays a vital role in brain glucose uptake and is highly expressed in cells forming the blood-tissue barriers and in astrocytes [37]. Glucose concentrations, specifically hypoglycaemia, induces upregulation of GLUT1 concentrations, while hyperglycemia does not seem to exhibit an effect [38,39]. Active mechanism for controlling sugar transport across the BBB and in astrocytes might be influenced by acute regulation of cell surface GLUT1 levels [40] and are potentially related to the energetic condition of brain tissue. Importantly, GLUT1 is not the sole glucose transporter at the BBB. The GLUT4 transporter for glucose seems to be expressed as well. GLUT3 is expressed in neurons and is likely providing glucose uptake into neurons, thus bypassing the glucose lactate shuffle through astrocytes by which lactate is provided as an energy rich substrate.

Another important aspect is brain protection against neuroactive substances such as aspartate and glutamate. The BBB is largely impermeable to these amino acids. Glutamate metabolisms by the liver provides for prompt transformation into glutamine and consequently, consumption of food containing high levels of glutamate such as tomatoes or food additives (e.g. soya sauce) does not affect brain function. Aspartate consumed by food is rapidly secreted through the kidney [41].

The limited transport of circulating monoamines through endothelial cells is attributed to the paracellular barrier capacity of tight junctions, the diffusional characteristics imposed by the lipid bilayer and to specific transport protein of the cell membrane. Intracellular levels of amino acids in the brain are correlated to the rates of influx across the BBB and synthesis of neurotransmitters such as serotonin, dopamine, and histamine are

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substrate limited [42]. For a sustainable supply of essential polar nutrients such as glucose and amino acids to the brain, there is a crucial role to specific solute carriers (SLC) across the BBB endothelium and all cells express a large number of SLCs in the cell membrane [43]. A wide variety of SLCs mediate the movement of nutrients and solutes in and out of the brain and are found on either the luminal or abluminal membrane only or inserted into both membranes of the endothelial cells. Detailed list of BBB SLCs are listed elsewhere (see Table 2 in Abbott et al., 2010).

3.2 Transport of ions

Preservation of an optimal environment for synaptic and neural function is achieved by specific ion channels and transporters. Water molecules can also cross the BBB through ion channels. Due to regulated ionic movement, potassium concentration in the CSF and brain interstitial fluid are maintained at ~ 2.5–2.9 mM despite the higher concentration of potassium in the plasma (~ 3.5-5.0 mM). In fact, potassium concentration can vary strongly during body exercise [44], nutrition or pathological conditions (Bradbury et al., 1963; Hansen, 1985) and may increase to levels as high as 10 mM in venous blood. If such increase in potassium concentrations would occur in the brain, a significant change in neuronal activity, specifically epileptic discharges, would be triggered. The BBB thereby protects the nerve cells from such variations. The BBB is similarly largely impermeable to most ions such as Ca^{2+} and Mg^{2+}. pH also is actively regulated at the BBB and the BCSFB [47,48]. The neurovascular unit and the BBB are also important in the spatial buffering of electrolytes upon neuronal activation. Astrocytes and their position between capillaries and neurons are connected with gap junctions, allowing them to communicate with each other and with capillary endothelial

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cells in contact with astrocytic processes. Neuronal firing and synaptic transmission are associated with the influx of Na^+ and Ca^{++} and the extracellular increase in the concentrations of K^+ and neurotransmitters. In addition, glucose metabolism during neuronal activity generates water at the rate of ~28 nl/g min^{-1} [49]. While neurotransmitters are recycled directly or via astrocytes, potassium is distributed spatially via astrocytes and water is excreted from the brain. Astrocytes have a key role in the homeostatic mechanisms maintaining brain extracellular environment within narrow limit, despite continuous neuronal activity, and the perivascular endfeet at the BBB have a particular role in these processes [50]. For example, extracellular K^+ ions accumulating during neuronal activity are expected to enter astrocytes according to the electrochemical gradient, and distributed to neighboring astrocytes (via gap junctions) and astrocytic endfeet. The high density of inwardly rectifying Kir4.1 on perivascular astrocytic endfeet makes them well suited for spatial buffering, depositing the K^+ in the perivascular space. The high density of AQP4 water channels in perivascular astrocytic endfeet facilitates a similar redistribution of water. Excess metabolic water may join the interstitial fluid in perivascular spaces and cleared through the cerebrospinal fluid. Similarly, the uptake of glutamate to astrocytes via specific transporters (mainly EAAT1 and 2) is Na^+-dependent and accompanied by net uptake of ions and water, that will similarly clear at the perivascular space and the BBB.

3.3 Transport of macromolecules- Proteins and Peptides

Endocytic vesicles account for the main delivery of large molecular weight substances such as proteins and peptides, through the BBB. Protein synthesis in the brain is dependent upon the supply of essential amino acids, most are neutral and large, thus incapable of passive diffusion to the brain. The typical concentrations of plasma proteins are higher than the CSF protein content, apparently due to the ability of the BBB to preclude the penetration of such macromolecules into the brain. Vesicular mechanisms involve either RMT or AMT enabling the transport of diverse large molecules and complexes. Summary of a number of known transcytotic mechanisms is presented elsewhere (Table 4 in Abbott et al., 2010). Internalization into the endothelial cell

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cytoplasm and exocytosis to the opposite pole of the cell occurs following interaction between ligands and cell-surface receptors that leads to caveolus and vesicle formation.

A possible mechanism for a peptide-specific transporter protein may facilitate their entrance through the membrane [51,52]. Growing body of evidence indicates that large molecular weight serum proteins infiltration though a dysfunctional BBB carries a potential risk for pathological outcomes within the CNS. Thrombin, plasmin and albumin were reported to induce local effects such as cellular activation, inflammation, apoptosis and epileptogenesis. The presence of some proteins in brain interstitial fluid can initiate signaling cascades resulting in seizures, activation of glia, synaptic plasticity and synaptogenesis and cell damage [53–55]. The wide presence and expression of Factor Xa (converting prothrombin to thrombin), tissue plasminogen activator (converting plasminogen to plasmin) and the thrombin receptor PAR1 likely play a role in these pathological pathways [56,57]. Albumin extravasations from the plasma into the brain milieu has been shown to be associated with astrocytic activation, activation of innate immune systems and the development of network modifications leading to epilepsy (David et al., 2009a; Ivens et al., 2007; Nadal et al., 1995 and see below).

3.4 Drug delivery

As mentioned above, the fact that penetration of large molecules from the blood into the brain is prevented by the BBB evokes an essential research and translational efforts aimed at development of novel treatments for many CNS pathologies and new radiopharmaceuticals for radio-labeled brain imaging techniques. A recent thorough review [60] provides an updated outline of classical modes of drug delivery to the brain, transcranial drug delivery or small molecules, endogenous carrier-mediated and receptor-mediated transport systems within the BBB and current approaches for reengineering of drugs to enable BBB transport.

3.5 Neuronal and vascular functions

A well-known observation is that the human brain, only ~2% of total body mass, consumes over 20% of total body oxygen and energy [61]. The term neurovascular coupling designates an integrated system of neuronal and vascular cells and their milieu working in concert to maintain brain homeostasis by providing the energy demands of

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neuronal activity via a tight, activity-dependent regulation on local blood flow. This complex process also involves pericytes, microglia, and specialized cellular compartments such as endothelial glycocalyx [62]. While the role of the intact BBB in controlling the normal neurovascular coupling is not completely understood, recent studies in injured patients hint that under conditions in which the BBB is severely impaired, vessels show impaired response to neuronal activation [63].

The BBB plays a crucial role in the maintenance of a strict extracellular environment around synapses and axons. Neurons interfaces within the CNS depend on chemical and electrical signals and thus a steady neural function is dependent upon the barrier capacity. Following BBB dysfunction the extracellular microenvironment is disturbed thus resulting in abnormal neuronal activity that may lead to seizures [64]. Sensory-motor neurological dysfunction developing after pathological vascular response and BBB opening, may be attributed to reduced metabolic efficacy, cellular damage and interference of homeostatic mechanisms such as active transporters and electrolyte buffering, required for neuronal activity [65]. As mentioned, the importance of intact BBB in maintaining the orchestrated relationship between brain activity and changes in blood flow is considered as a key determinant in the pathophysiology of brain injuries. While neurovascular coupling may reflect a physiological homeostatic response to increased metabolic demand, recent animal and human data suggest that reduced energy supply and worsening of the tissue metabolic state will promote cellular damage and slow energy-demanding homeostatic mechanisms as active transporters required for neuronal repolarization. As reviewed by Dreier [66], under pathological conditions, the physiological neurovascular coupling may fail, and neuronal depolarization during seizures or spreading depolarization may be associated with no or “inverse coupling” – i.e. vasoconstriction.

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Figure 1:

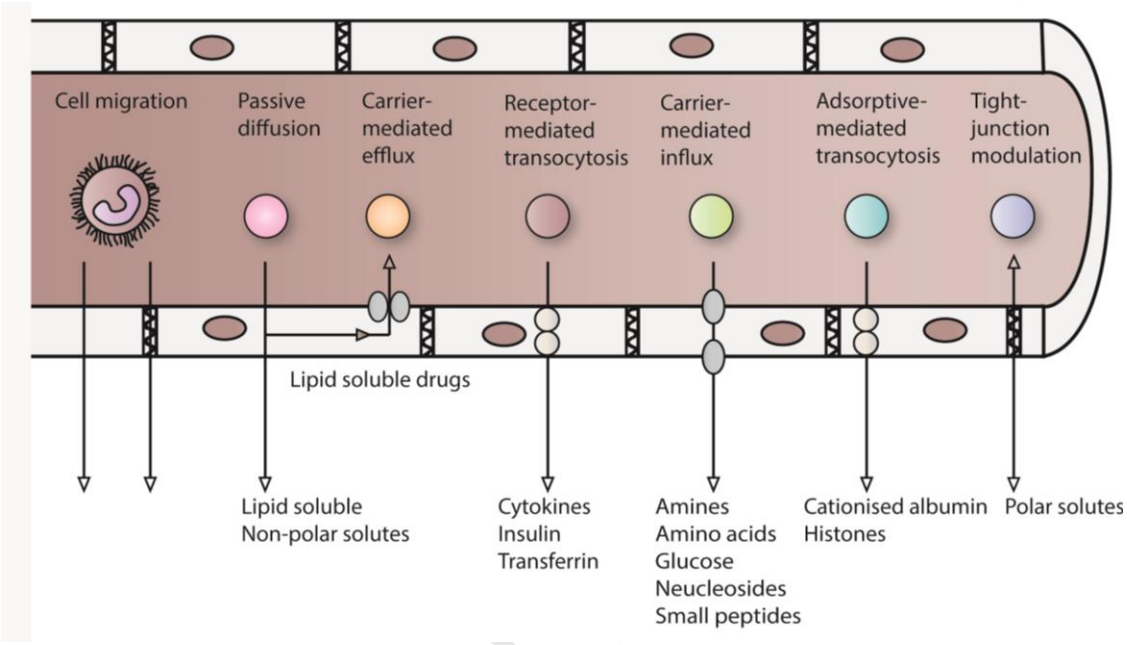

Figure legend:

Potential routes for infiltration and transport across the endothelial cells forming the BBB; Cells may cross the BBB through or adjacent to the tight junctions. Solutes may passively diffuse through the cell membrane. Active efflux carriers may pump some of these passively penetrating solutes out of the endothelial cell. Carrier-mediated influx (passive or secondarily active) can transport essential polar molecules, such as amino acids, glucose and nucleosides into the CNS. Receptor-mediated transcytosis (RMT) can transport macromolecules such as peptides and proteins across the endothelium. Adsorptive-mediated transcytosis (AMT) is induced non-specifically by positively charged macromolecules and can result in passage across the BBB. Tight junction modulation may occur, affecting the permeability of the paracellular aqueous diffusional pathway.