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Blood-Brain Barrier Permeability: From Bench to Bedside

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1. Introduction

The concept of the blood-brain barrier (termed hematoencephalic barrier) was first introduced by Lina Stern in 1921, although the early work by Paul Ehrlich and Edwin Goldmann suggested the compartmentalization between blood and brain and a role of blood vessels in maintaining these compartments (Ehrlich, 1885; Goldmann, 1913; Vein, 2008). However, actual proof of the existence of a BBB came in the 1960s. Since then, significant progress has been made in defining the functions and properties of that barrier.
The BBB is a highly specialized structural and biochemical barrier that regulates the entry of blood-borne molecules and cells into brain and preserves ionic homeostasis within the brain microenvironment (Pardridge, 2007; Rubin & Staddon, 1999; Ueno, 2007). Formed at the interface between blood and brain parenchyma, the BBB is composed of a tightly sealed monolayer of brain endothelial cells at the brain capillary surface and adjacent perivascular cells, including astrocytes and pericytes. Both astrocytic endfeet and pericyte processes wrap the abluminal capillary surface and through indirect or direct synapse-like "peg-socket" interactions provide physical support and stability to the BBB (Abbott, 2002; Armulik et al, 2010; Kim et al, 2006; Williams et al, 2001). In recent years, the concept of a BBB has been significantly extended to the concept of a neurovascular unit, which best describes the dynamic communication between brain endothelium, neurons, astrocytes, pericytes, vascular smooth muscle cells, microglia and perivascular macrophages at the interface between the blood and brain parenchyma compartments (Hawkins & Davis, 2005; Wolburg et al, 2009). A healthy brain relies on all of the cells of the neurovascular unit to function properly and communicate with each other in order for neuronal synapses and circuitries to maintain normal cognitive functions (Fig. 1).

2. Blood-brain barrier junctional complexes

The structural properties of the BBB are primarily determined by the endothelial junctional complexes, consisting of tight junctions (TJ) and adherens junctions (AdJ). The interactions between brain endothelial cells provide high endothelial electrical resistance barrier, in the range of 1500-2000 Ω.cm^2 (pial vessels), as compared to 3-33 Ω.cm^2 endothelial barrier in

other tissues (Butt et al., 1990; Crone & Christensen, 1981). The TJ complexes seal the interendothelial cleft and regulates BBB paracellular permeability, while the AdJ is important for initiating and maintaining endothelial cell-cell contact (Denker &Nigam, 1998; Huber et al, 2001; Gonzalez-Mariscal et al, 2003). Structurally both complexes are composed of transmembrane proteins, which physically interact with their counterparts on the plasma membrane of adjacent cells, and cytoplasmic plaque proteins, which provide a link between transmembrane TJ/AdJ proteins and the actin cytoskeleton and participate in intracellular signaling (Fig. 2).

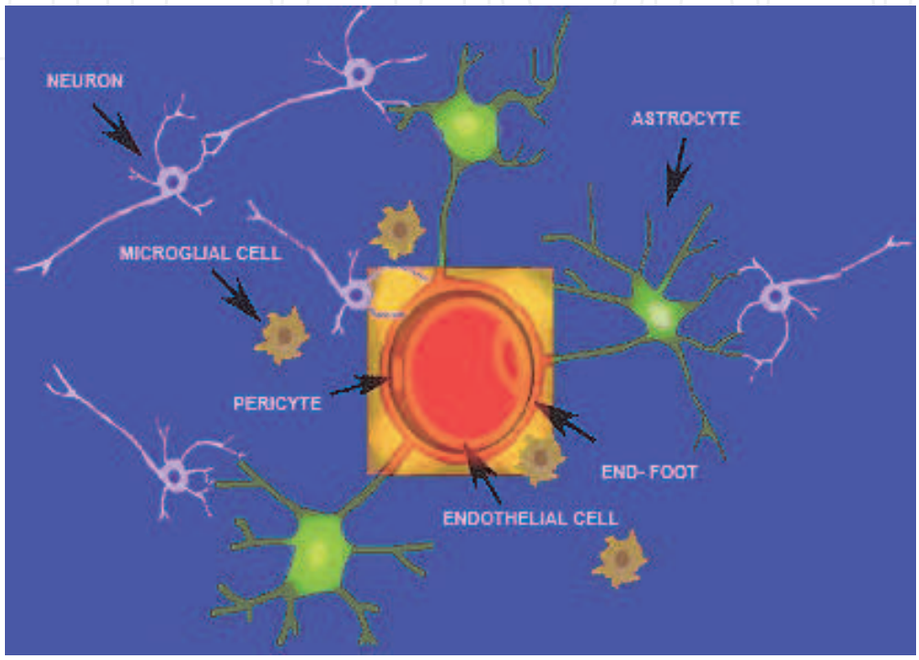

Fig. 1. Blood Brain barrier: neurovascular units.

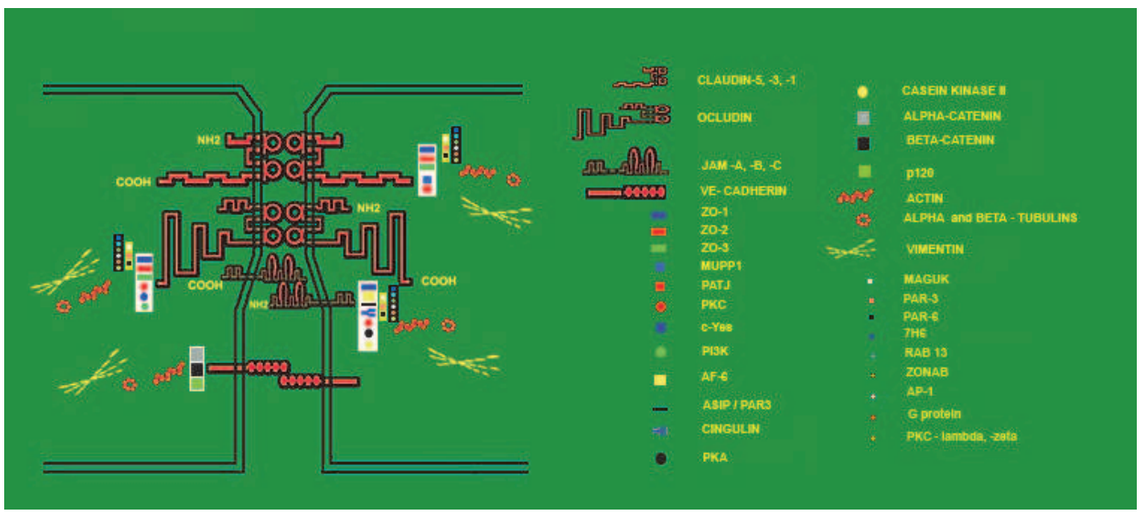

Fig. 2. Blood brain barrier: Tight and adherenst junction complex

The TJ transmembrane proteins include occludin, claudins (for example, claudin-5, -3, -12, -1) and junctional adhesion molecule (JAM) -A, -B and -C (Martin-Padura et al, 1998; Mitic & Aderon, 1998; Staddon and Rubin, 1996). Occludin (MW ~65kDa) was one of the first TJ transmembrane proteins to be described. It has four transmembrane spanning regions, two extracellular loops responsible for intercellular adhesion and maintaining transendothelial electrical resistance, and N- and C- terminal sites through which occludin can fully oligomerize or directly interact with scaffolding TJ [zonula occludens -1, -2, -3 (ZO-1, -2- 3)] and regulatory proteins [protein kinase C (PKC), tyrosine kinase c-Yes and Phosphatidylinositol 3-kinases (PI3K)] (Clump et al, 2005; Feldman et al 2005; Suzukiet al, 2002). The C-terminus of occludin plays a critical role in paracellular channel formation, mediating endocytosis and trafficking of occludin (Li et al, 2005; Nusrat et al, 2005). It is also involved in the integration and function of occludin within the TJ complex.

Claudins (MW 20 to 27 kDa) are the principal barrier-forming proteins. They belong to the PMP22/EMP/MP20/claudin family of proteins (Koval, 2006). Until now, twenty different claudins have been discovered and each of them shows a unique pattern of tissue expression and interactions. Claudins have a similar structural pattern to occludin: four membrane-spanning regions, two extracellular loops and two cytoplasmic termini (Morita et al, 1999; Nitta et al, 2003; Ruffer & Gerke, 2004; Soma et al, 2004). The first extracellular loop influences paracellular charge selectivity, while the second loop is known as a receptor for a bacterial toxin. Similar to occludin, the C-terminal site of claudins possesses a binding site (domain) for cytoplasmic proteins (ZO-1, ZO-2, ZO-3, MUPP1, PATJ) through a PDZ motif (Koval, 2006; Morita et al, 1999; Ruffer & Gerke, 2004). The role of the N-terminal site is still unclear. Brain endothelial cells express the cell specific claudin-5, which plays pivotal role in interendothelial occlusion and size selective permeability (Nitta et al, 2003; Ohtsuki et al, 2007). Besides claudin-5, recent data suggest the BBB possesses claudin-3, mostly during vasculogenesis, claudin-1, during adult brain angiogenesis and barrier genesis, and claudin-12 (Belanger et al, 2007; Lampugnani et al, 2010). However, there is little information on the interaction between these claudins and their role at the BBB.

JAM-A, -B, and -C (MW 32 kDa) are members of the immunoglobulin superfamily of proteins (Martin-Padura et al 1998). Similar to other immunoglobulins, these molecules are composed of a single membrane spanning domain, an extracellular domain, and two termini, an extracellular N-terminus and a short cytoplasmic tail C-terminus (Sobocki et al, 2006; Williams et al, 1999). The extracellular region of JAMs consists of two IgG-like domains and it appears to be subject to glycosylation, although the function of that glycosylation is still unknown. The short cytoplasmatic tail (40 amino acids) contains a binding domain which facilitates interactions with TJ associated scaffold proteins such as ZO-1, AF-6, ASIP/Par3, and cingulin (Bazzoni et al 2000; Bazzoni & Dejana, 2004; Williams et al, 1999). It also has phosphorylation sites for PKC, PKA and casein kinase II (Williams et al, 1999). JAMs display different patterns of homophilic and heterophilic cis- and trans-interactions. While they interact with JAM on adjacent cells, they can also act as adhesion molecules for interacting with integrins on leukocytes to regulate leukocyte trafficking (Bazzoni et al, 2000; Lamagna et al, 2005).

The cytoplasmic plaque proteins of TJ are divided into PDZ containing proteins (family membrane-associated guanylate-kinase (MAGUK) homologues (ZO-1, ZO-2, ZO-3), partitioning-defective proteins Par-3, Par-6, afadin/Af-6) and PDZ lacking proteins (cingulin, 7H6, Rab13, ZONAB, AP-1, PKCζ, PKCλ, heterotrimeric G protein) (Gonzalez-Mariscal et al, 2000, 2003; Ponting et al, 1999). The PDZ containing TJ proteins act as

scaffolds that bring together cytoskeleton, signaling and integral proteins at specific regions of the plasma membrane and, via the PDZ domain, have a critical role in clustering and anchoring transmembrane proteins (Fanning et al, 2007; Hamazaki et al, 2002; McNeil et al, 2006). For example, ZO-1 functions as a multidomain scaffold that coordinates assembly of transmembrane and cytosolic proteins, including components of the cortical cytoskeleton, into TJs and/or regulates the activity of these proteins once they are assembled. Thus, ZO-1 is required for the normal kinetics of TJ assembly, for TJ specific localization and unique organization of transmembrane proteins (Gonzalez-Mariscal et al, 2000; McNeil et al, 2006; Utepbergenov et al, 2006).

PDZ lacking proteins have a variety functions at the TJ complex. For example, cingulin acts as a cross-link between TJ proteins (ZO-2, ZO-3, AF-6, JAM) and the actin-myosin cytoskeleton. Rab proteins (Rab13, Rab3b) have a role in the docking and fusion of transport vesicles at the TJ complex, while PKCζ and PKCλ have roles in regulating polarization and in TJ assembly (Andreeva et al, 2006; Suzuki et al, 2002; Terai et al, 2006; Yamanaka et al, 2001;). G proteins (Gα-i0, Gα-i2, Gα12, Gαs) co-immunoprecipitate with ZO-1 and play a role in accelerating TJ assembly and maintaining transendothelial electrical resistance (Citi &Cordenonsi, 1998; Meyer et al, 2003).

The major AdJ transmembrane protein in endothelial cells is vascular endothelium (Ve)-cadherin. The AdJ cytoplasmic plaque proteins include catenin family members (α-, β-catenin, p120) (Bazzoni & Dejana, 2004; Nagafuchi, 2001). Ve-cadherin is an important determinant of microvascular integrity both *in vitro* and *in vivo*. Together with the catenins, it forms a complex that functions as an early recognition mechanism between endothelial cells (Vorbrodt & Dobrogowska, 2004). In that complex, β-catenin and p120 are linked with cadherin and to α-catenin, and this provides a functional interaction for Ve-cadherin with the actin microfilament network of the cell cytoskeleton. A p120 binds Ve-cadherin with high affinity suggesting that it may be engaged in regulating vascular permeability (Hatzfeld, 2005; Tao et al, 1996; Vorbrodt & Dobrogowska, 2004).

The actin cytoskeleton is also a critical component for establishing brain endothelial barrier integrity. The cytoskeleton is composed of three primary elements: actin microfilaments, intermediate filaments and microtubules. Actin microfilaments are focally linked to multiple membrane adhesive proteins such as cadherin, occludin, zonula occludens, catenins and focal adhesion complex, forming a structure known as the actin-rich adhesion belt and providing physical support to the junctional complexes (Lai et al, 2005; Small et al, 1999; Tao et al, 1996). In addition, actin microfilaments are involved in generating tension via myosin light chain phosphorylation and actin stress fiber formation during the unsealing of the junctional complex (Small et al, 1999; Stamatovic et al, 2003; Wang et al, 1983). A second major element of the cytoskeleton is the microtubules, polymers of α- and β-tubulins, which participate in rapid assembly of actin filaments and focal adhesion, isometric cellular contraction and/or increased transendothelial leucocyte migration (Honore et al, 2005; Tzima, 2006). A third major element of the cytoskeletal machinery is the intermediate filaments (predominantly vimentin) which have a role in reorganization of actin filaments and microtubules (Dudek& Garcia, 2001).

3. Blood-brain barrier transport systems

3.1 Transcellular transport

Due to the restrictive angioarchitecture of the BBB, brain endothelial cells have developed specific transport systems which allow the controlled exchange of proteins, nutrients and

waste products between blood and brain. In this way, while impeding the general influx of hydrophilic intravascular substances from blood to brain, carrier- and receptor-mediated transport systems promote the transport into brain of select compounds important for cerebral function. In addition, active efflux transport systems promote the clearance of select compounds (e.g. waste products) from brain to blood. There is some 'non-selective' transport of compounds across the BBB through nonspecific vesicular transport (fluid phase endocytosis or adsorptive endocytosis) (Lossinsky et al, 1983; Lossinsky & Shivers, 2004).

Fluid phase endocytosis, adsorptive endocytosis and caveolae are some of the systems involved in the transcytosis of compounds across the brain endothelium. Transcytosis describes the vectorial movement of molecules within endocytotic vesicles across the cerebral endothelium (primarily from the luminal cell side to the abluminal side) where exocytosis occurs (Lossinsky & Shivers, 2004). Brain capillary endothelial cells contain two kinds of vesicles that are open to the luminal blood capillary space: caveolae and clathrin-coated pits/vesicles. Clathrin is a self-assembling protein whose polymerization into a polyhedral network promotes membrane vesiculation and budding of selected receptors (Miwako et al, 2003; Mukherjee et al, 1997). Compared to peripheral endothelia, the brain capillary endothelium is particularly enriched in clathrin-coated pits/vesicles (Lossinsky & Shivers, 2004). These are predominantly expressed on the luminal side suggesting that clathrin-dependent transcytosis is primarily from blood to brain. Clathrin-coated pits recruit cell-surface receptors and then, through a series of highly regulated steps, pinch off to form clathrin-coated vesicles, which further may fuse with a transcytotic endosome (Miwako et al, 2003; Mukherjee et al, 1997).

Caveolae are bud-like invaginations formed by the concentration of the caveolin proteins. These vesicles are enriched in cholesterol and glycosphingolipids on cellular membranes as well as glycosyl phosphatidyl inositol (GPI)-anchored proteins, not present in the coated pits (Hommelgaard et al, 2005; Kirkham & Parton, 2005). Caveolae are found on both luminal and abluminal plasma membranes of cerebral endothelial cells indicating bidirectional transcytosis from blood to brain and from brain to blood (Lossinsky & Shivers, 2004). Caveolae contain an abundance of membrane receptors, transporters and signaling molecules, suggesting their involvement in various important cellular processes in addition to their role in the endocytosis/transcytosis. Recent findings regarding the process of endocytosis have pinpointed the merging endosomes for both types of endocytotic pathways (see for review Hommelgaard et al, 2005) .

In fluid-phase transcytosis, invagination of caveolae entraps bulk plasma and soluble plasma molecules. The vesicles are then transported across the cerebral endothelium. In this transport process, there is a lack of interaction between the transported molecules and the caveolar vesicular membrane (Lossinsky & Shivers, 2004; Predescu et al, 2007). A very small portion fluid-phase transcytosis can occur via clathrin-coated pits/vesicles.

Adsorptive transcytosis can be *specific* (receptor-mediated transcytosis) and *nonspecific* (adsorptive-mediated transcytosis) processes. Receptor-mediated transcytosis is triggered by a specific interaction of a molecule with receptors expressed on capillary brain endothelial cells and it is limited to transport of proteins and peptides across the BBB. Examples of this type of adsorptive transcytosis are insulin, iron-transferrin and LDL-cholesterol (Broadwell et al, 1996; Hervé et al, 2008; Simionescu & Simionescu, 1985) This type of transport is very limited in brain endothelium with small amounts of insulin and transferrin being delivered into brain sufficient to maintain BBB and brain homeostasis. Clathrin-type vesicles are predominantly involved in receptor-mediated transcytosis.

Non-specific adsorptive transcytosis does not involve specific plasma membrane receptors and endocytosis is initiated through charge-charge interaction between polycationic substances and negative charges on the endothelial surface. Molecules that penetrate the brain via this mechanism include, but are not limited to, various cationic proteins. Clathrin-coated pits along the luminal surface of ECs are negatively charged and thus capable of binding positively charged substances (Hervé et al, 2008; Villegas et al, 1993). A few studies have, however, demonstrated that caveolae are involved in adsorptive endocytosis by transferring of cationized $F(ab')_2$ antibody fragments across the BBB (Girod et al, 1999).
Brain uptake via non-specific and specific adsorptive transcytosis is time- and concentration-dependent, and requires energy. Uptake via these types of endocytosis is slow compared with carrier-mediated transport of nutrients (e.g. glucose), taking minutes to occur. Both non-specific and specific adsorptive endocytosis/transcystosis are also saturable processes with the main difference being that non-specific adsorptive transcytosis becomes saturated at higher concentrations (micromolar level) while specific adsorptive transcytosis becomes saturated at a low nanomolar range (Hervé et al, 2008).

3.2 Carrier mediated: blood-to-brain influx systems

BBB possesses a wide array of carrier-mediated transport systems for small molecules to support and protect CNS function. For example, the blood-to-brain influx transport systems supply nutrients, such as glucose and amino acids.
D-glucose in the primary energy source for the brain and the BBB has very high levels of the facilitative (Na^+-independent) glucose transporter, GLUT1 (SLC2A1) which transports D- but not L-glucose (Cornford et al, 1993; Pardridge et al, 1990). GLUT1 is localized on both the luminal and abluminal sides of the BBB. As well as transporting D-glucose, GLUT1 transports hexoses and an oxidized form of L-ascorbic acid, L-dehydroascorbic acid. It is considered to have role in maintaining the high concentration of L-ascorbic acid in the brain compared with plasma (McAllister et al 2001; Vemula et al, 2009). In addition, GLUT1 can transport some glycosylated peptides (e.g. L-serinyl-β-D-glucoside analogues of Met^5 enkephalin) (Masand et al, 2006).
Amino acids like L-tyrosine, L-tryptophan, and L-histidine are transported from the blood to the brain via a Na^+-independent neutral amino acid transporter (system L) (Boado et al, 1999, Ohtsuki, 2004). This is a heteromeric transporter with a light chain (LAT1; SLC7A5) and a heavy chain (4F2hc; SLC3A2)(Omidi et al, 2008). As with GLUT1, it is facilitative and present on both the luminal and abluminal membranes. Same transporters is involved in transports L-leucine, L-isoleucine, L-valine, L-methionine, L-threonine, and L-phenylalanine (Audus & Borchardt, 1986; Omidi et al, 2008; Reichel et al, 1996; Xiang et al, 2003). Several amino acid-mimetic drugs, alkylating agent melphalan, the antiepileptic drug gabapentin, and the muscle relaxant baclofen use a System L for the influx form blood to brain (Luer et al, 1999; Sakaeda et al, 2000). Thus a high-protein diet reduces the concentration of these drugs in the brain due to competitive inhibition at the BBB.
The basic amino acids, such as L-lysine and l-arginine have a CAT1 (SCL7A2) transporter which expression is concentrated in brain capillaries (Lyck et al, 2009; Umeki et al, 2002). TAUT (SLC6A6) mediates taurine transport at the BBB, and due to neuroprotective effect of taurine the therapeutic manipulation of this transporter is important strategy in the treatment of neurodegenerative disorders (Kang et al, 2002; Lyck et al, 2009).
MCT1 (SCL16A1) mediates influx transport of monocarboxlic acids, such as lactate and pyruvate. MCT1 in the brain and the brain uptake rate of lactate are particularly increased

during the suckling period allowing brain the use lactate from milk (Batrakova et al, 2004; Kido et al 2000; Umeki et al, 2002).
CNT1 (SCL28A1) mediates transport of nucleosides and their analogues while Oatp2 (SLCO1B1; SLC21A6) mediates transport of organic anions and opioids (Bourasset et al, 2003; Cansev, 2006; Gao et al, 2000; Li et al, 2001). Both of these blood-to-brain influx transport systems are candidates to enhance drug delivery to the brain.
Creatine is important in energy storage in the brain and it is uptaken via CRT [SLC6A8] transporter (Braissant et al, 2001; Ohtsuki et al, 2002; Tachikawa et al, 2009;). This transporter is expressed on both luminal and an abluminal membrane of brain capillary endothelial cells and for this transporter is documented to mediate creatine supply to the brain (Braissant et al, 2001; Ohtsuki et al, 2002). Due to the fact that creatine has a neuroprotective effect, targeting CRT at BBB is the strategy to increase brain creatine levels and to prevent neurodegeneration (Fig. 3).

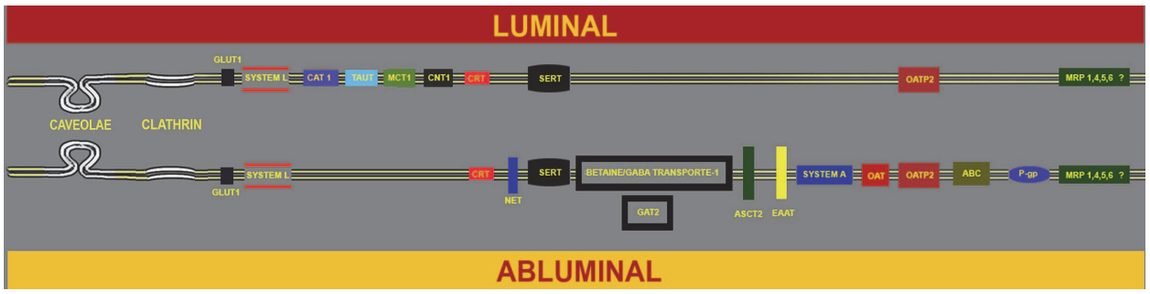

Fig. 3. Blood brain barrier transport system.

3.3 BBB efflux transporters: brain-to-blood efflux system

The BBB is involved in the brain-to-blood efflux transport of hydrophilic small molecules generated in the brain, such as neurotransmitters, neuromodulators, end-metabolites of neurotransmitters, uremic toxins, and also peptides, such as immunoglobulins.
Brain endothelial cells contain the norepinephrine transporter (NET), localized at the abluminal membrane and serotonin transporter (SERT), localized at both the abluminal and luminal membrane. In this way the brain microvasculature could receive signals and be regulated by monoamines released from adrenergic and serotonergic neurons (Ohtsuki, 2004; Wakayama et al, 2002). The abluminally localized NET and SERT is thought to be an inactivation system for neurotransmitters around the brain capillaries. The presence of luminal SERT is thought to play a role in serotonin clearance from the intravascular space (mostly secreted by platelets) to maintain cerebral blood flow (Nakatani et al, 2008; Olivier et al, 2000). Besides monoamines, brain endothelial cells are also involved in the efflux transport of GABA via Betaine/GABA transporter-1 (BGT-1; SLC6A12) or murine GABA transporter 2 (GAT2) present on the abluminal membrane (Gibbs et al, 2004; Kakee et al, 2001; Takanaga et al, 2001).
Brain endothelial cells exhibit stereo-selective efflux transport of aspartic acid (Asp), via ASC transporter ASCT2, selectively transporting the L-isomer of Asp (Tetsuka et al, 2003). In addition, excitatory amino acid transporters, EAATs, have been detected on the abluminal membrane of brain endothelial cells having a role in transport of both L- and D-Asp isomers and L-glutamate (Ennis et al, 1998; O'Kane et al, 1999; Tetsuka et al, 2003). System A is a transport system (ATA1, ATA2, ATA3) for small neutral amino acids that

accepts L-alanine, L-proline and glycine (Hatanaka et al, 2001; Ling et al, 2001). Present on the abluminal membrane of brain endothelial cells, this system also may contribute to the regulation of the osmolarity in the brain and cell volume (Hatanaka et al, 2001; Ohtsuki, 2004).

The organic anion transporter (OAT) family is also involved in efflux transport at the BBB. These transporters are involved in the efflux of various neurotransmitter metabolites and act as a CNS detoxification system (Ohtsuki et al, 2004). For example, OAT3 (SLC22A8), localized at the abluminal membrane, transports homovanillic acid (HVA) from brain to blood (Mori et al, 2003; Ohtsuki et al, 2002). OAT3-mediated HVA transport is inhibited by various neurotransmitter metabolites such as 3,4-dihydroxyphenylacetic acid (dopamine metabolite), vanillylmandelic acid, 3,4-dihydroxymandelic acid and 4-hydroxy-3-methoxyphenylglycol (norepinephrine and epinephrine metabolites), 5-hydroxyindole acetic acid and 5-methoxytryptophol (serotonin metabolites), and imidazole-4-acetic acid and 1-methyl-4-imidazolic acid (histamine metabolites) (Duan & Wang, 2010; Ohtsuki et al, 2002). Thus it appears that OAT3 mediates the clearance of a wide range of neurotransmitter metabolites from brain. In addition, OAT3 mediates the brain-to-blood efflux of indoxyl sulfate, a uremic toxin (Ohtsuki et al, 2002). The brain concentration of under normal conditions is 3.4 times lower than that in serum and this limited distribution could be due to OAT3-mediated BBB efflux (Ohtsuki et al, 2002).

Another transporter of organic anions, Oatp2, is localized on both the luminal and abluminal membrane of brain endothelial cells and plays a role in the efflux of dehydroepiandrosterone sulfate, a neurosteroid that can interact with GABA type A receptors and σ receptors to increase memory and learning ability and to protect neurons against excitatory amino acid-induced neurotoxicity (Asaba et al, 2000; Gao et al, 1999; Ose et al, 2010). Oatp2 is also responsible for estrone-3-sulfate efflux transport (Asaba et al, 2000).

BBB active drug efflux transporters know as ATP-binding cassette (ABC) efflux transporters are increasingly recognized as important determinants of drug distribution to, and elimination from, the brain, minimizing or avoiding in this way neurotoxic adverse effects of drugs that otherwise would penetrate into the brain (Begley, 2004). Until now the best characterized of the BBB ABC efflux transporters are P-glycoprotein (Pgp, ABCB1), the multidrug resistance associated protein MRP (ABCC2) family and breast cancer resistance protein (BCRP) (Eisenblätter et al, 2003; Virgintino et al, 2002; Zhang et al, 2003).

P-glycoprotein (P-gp/MDR1/ABCB1) is a well-characterized efflux transporter of xenobiotics (Löscher et al, 2005). P-gp is a primary active transporter of relatively lipophilic compounds, such as the anticancer drug, vinblastine, cyclosporin A, and the cardiac glycoside, digoxin, by direct consumption of ATP (Hembury et al, 2008; Löscher et al, 2005; Quezada et al, 2008; van der Sandt et al, 2001). In addition, P-gp contributes to efflux of such as amyloid-beta proteins from the brain into the blood as well as many drugs such as anti-cancer drugs (Cirrito et al, 2005; Nazer et al, 2008; Piwnica-Worms et al, 2006). P-gp expressed on the luminal side of brain endothelial cells plays a very important role in restricting the entry of xenobiotics from the circulating blood into the brain (Matsuoka et al, 1999, Warren et al, 2009). Thus, for example, ivermectin reaches 20-fold higher concentrations in the brains of mice without P-gp (Lespine et al, 2006).

The multidrug resistance-associated protein (MRP) 1, 4, 5, and 6 has been detected in primary cultured bovine brain endothelial cells and the bovine brain capillary-enriched fraction (Nies et al, 2004; Yu et al, 2007; Zhang et al, 2000). MRP1 and 5 are predominantly

localized on the luminal membrane fraction while MRP4 is localized almost equally on the luminal and abluminal membrane fractions (Nies et al, 2004; Yu et al, 2007). However, the localization of these subtypes is still unclear. Although the full functions of MRPs are still unknown (and the relative importance still debated), one recent study indicated that Mrp1 contributes in part to the efflux transport of Estradiol-17-β-D-glucuronide ($E_2$17βG) at the BBB (Sugiyama et al, 2003) (Fig. 3).

4. BBB and epilepsy

Epilepsy is a chronic neurological disease that is characterized by spontaneous recurrent seizures and sometimes-untreatable seizures. In addition, epileptogenesis can occur after brain insults such as trauma, ischemia and infection. Several clinical and experimental studies have reported that BBB malfunction can trigger chronic seizures or an acute seizure (Friedman et al, 2009; Oby & Janigro, 2006; Tomkins et al 2011). Furthermore, transient BBB disruption is a consequence of epileptic seizures and multiple changes in BBB transporters have been reported in epilepsy patients/models. BBB obviously play an important multifaceted role in epileptic seizures as discussed below (Dombrowski et al, 2001; Löscher et al, 2002; Łotowska et al, 2008).

Pathological and immunohistochemical studies in human epileptic tissue as well as animal models of epilepsy consistently demonstrate structural evidence for an abnormal "leaky" BBB with an accumulation of serum albumin within the neuropil and cellular elements as functional evidence for abnormal vessels permeability to large hydrophilic molecules (Oby & Janigro, 2006; Stewart et al, 1987). A substantial increase in BBB permeability was found in approximately 2/3 of capillaries and perivascular astroglial processes.

4.1 Blood Brain Barrier permeability and epilepsy

Increased BBB permeability is associated with remodeling of interendothelial junctional complex and gap formation between brain endothelial cells (paracellular pathway) and/or intensive pinocytotic vesicular transport between the apical and basal side of brain endothelial cells (transcellular pathway) (Bazzoni, 2006; Garcia & Schaphorst, 1995; Lossinsky & Shivers, 2004). These two pathways display differences in cellular and molecular components as well as in physical properties. The transcellular pathway can be either passive or active, and is characterized by low conductance and high selectivity in either apical to basal or basal to apical directions. In contrast, the paracellular pathway is exclusively passive, being driven by electrochemical and osmotic gradients, and it shows a higher conductance and lower selectivity, although it can display charge and size selectivity (Bazzoni, 2006). There is evidence that both types of pathway are involved in the development and progression of epilepsy seizures. Ultrastructural studies on human epileptic tissue clearly demonstrated BBB abnormalities, including increased micropinocytosis and fewer mitochondria in endothelial cells, a thickening of the basal membrane, and the presence of abnormal tight junctions (Cornford & Hyman, 1999; Cornford & Oldendorf, 1986).

Increased BBB permeability could be an etiological factor contributing to seizure development. Both clinical and animal studies pinpoint that primary vascular lesions and, specifically an opening of the BBB (i.e. significant and long-lasting BBB breakdown in cortical injury), trigger a chain of events leading to epilepsy (Marchi et al, 2007; Oby and Janigro, 2006; Seiffert et al, 2004; Tomkins et al, 2007; Tomkins et al, 2008; van Vliet et al,

2007). Increased BBB permeability was found in 77% of patients with posttraumatic epilepsy and these patents had a larger cerebral cortex volume with BBB disruption (Tomkins et al, 2008). In 70% of patients, slow (delta band) activity was co-localized, by sLORETA, with regions showing BBB disruption (Tomkins et al, 2011). A consequence of increased para- and transcellular permeability is extravasation of albumin into the brain neuropil. This may be sufficient for the induction of epileptogenesis. It has been suggested that accumulated albumin binds to transforming growth factor beta receptor 2 (TGFbetaR2) in astrocytes and induces rapid astrocytic transformation and dysfunction (Cacheaux et al, 2009; David et al, 2009; Ivenset al, 2007;) In addition, leakage of some other serum-derived components into the extracellular space may also result in hyperexcitability and seizure onset. For example, it has been recently shown that the serum protein, thrombin, via receptors protease-activated receptor 1 (PAR1), produces a long-lasting enhancement of the reactivity of CA1 neurons to afferent stimulation (Maggio et al, 2008). It should also be noted that in many cases of epilepsy, that BBB breakdown has been associated with early or delayed neuronal damage (Rigau et al, 2007; Tomkins et al, 2007; van Vliet et al, 2007).

Furthermore, BBB dysfunction may not only trigger epileptic seizures, it may also contribute to the progression of epilepsy (Seiffert et al, 2004, van Vliet et al, 2007; Uva et al, 2008). Recently, a role for BBB opening in the progression of temporal epilepsy was suggested based on the finding of positive immunocytochemistry staining for accumulated albumin and a positive correlation between the extent of BBB opening and the number of seizures (van Vliet et al, 2007). In the line with that evidence, application of bile salts causes long-lasting BBB opening caused by application of bile salts and the delayed appearance of robust hypersynchronous epileptiform activity (Greenwood et al, 1991). Predictors of seizures during the BBB breakdown are elevation of serum S100beta (an astrocyte marker) levels and computed tomography (CT) scans (Marchi et al, 2007).

Vasogenic brain edema is one the best example of association between BBB dysfunction and epilepsy. In experimental epilepsy models (kainate- and pilocarpine-epilepsy models, layers II and III of the piriform cortex are vulnerable to brain edema and they have been shown to play a role in generation and propagation of paroxysmal activity (Gale, 1992, Löscher and Ebert, 1996, McIntyre and Kelly, 2000). In contrast to the piriform cortex, the hippocampus shows vacuolized CA1 astrocytes and neuronal death without vasogenic edema (Kim et al, 2009, Kim et al, 2010).

Many studies have reported an increased permeability of the BBB during epileptic activity (Öztas and Kaya, 1991, Ruth, 1984; Ilbay et al, 2003, Ates et al, 1999). A fast and significant increase in systemic blood pressure, particularly during tonic epileptic seizures, induces marked vasodilation of large cerebral arteries and an increase in blood pressure in capillaries, small arteries, and veins, leading to leakage of the BBB (Mayhan, 2001). Indeed, an acute increase in blood pressure or epileptic activity causes increased pinocytosis in the cerebral endothelium (Cornford and Oldendorf, 1986).

The loss of BBB integrity, however, is not only due to an abrupt increase in intraluminal pressure but also influenced by the properties of cerebral tissues, particularly in the perivascular area (Nitsch et al, 1985). The most notable changes are on perivascular astrocytes. Several recent studies have pinpointed alterations in astrocytic dystrophin expression during epileptogenesis, which may directly influence brain endothelial barrier permeability. Dystrophin, an actin-binding protein, is primarily localized in the astrocyte end-feet near capillaries where this anchor protein is responsible for maintenance of polarized expression of astrocytic aquaporin 4 (AQP4; a water channel) (Nico et al, 2003;

Sheen et al, 2011). Since astrocytes selectively control exchange between blood and neural tissue by induction of the morphological and biochemical features of endothelial cells to form TJ and of its enzymatic systems and transporters, it is likely that dystrophin plays a role in establishment of endothelial polarity and regulating vascular permeability (Anderson et al, 1989; Nico et al, 2003). However, some dystrophin isoforms are also present in brain endothelial cells where, as an actin binding protein, dystrophin may regulate the actin machinery associated with the TJ complex (Nico et al, 2004). During epileptogenesis, there is down-regulation of dystrophin immunoreactivity in perivascular astrocytes and endothelial cells and this was also accompanied by impaired AQP4 expression in perivascular astroglial end-feet. The perturbed expression of AQP4 and dystrophin furthermore may be one factor underlying loss of ion and water homeostasis in the epileptic brain tissue, leading to impaired buffering of extracellular K^+ and an increased propensity for seizures (Lee et al, 2004, Eid et al, 2005).

SMI-7 is an endothelial barrier antigen expressed on the luminal membrane at the rat BBB (Sternberger & Sternberger, 1987). SMI-71 immunoreactivity is also significantly reduced in blood vessels at day 1 after epileptogenesis when the neuronal damage is also present (Lu et al, 2010). However, the down-regulation of SMI-71 is also associated with widening of intercellular junctions between endothelial cells and swelling of perivascular astrocytic processes and this was caused by impaired interaction between endothelial cells and perivascular astrocytes (Ghabriel et al, 2002; Lawrenson et al, 1995).

4.2 Angiogenesis, blood brain barrier and epilepsy

In humans, there is evidence that cerebral vascularization (vessel density) is significantly higher in temporal lobe epilepsy (Rigau et al, 2007). This was neither dependent on etiology nor on the degree of neuronal loss, but was positively correlated with seizure frequency. Vascular endothelial growth factor (VEGF) and the VEGF receptors were detected in different types of cells suggesting a role of this growth factor in the increased vascularization. In that study, an impairment of the BBB was demonstrated by a loss of TJs and by immunoglobulin G (IgG) leakage and accumulation in neurons. In a rat model of temporal lobe epilepsy, VEGF over-expression and BBB impairment also occurred early after status epilepticus (Croll et al; 2004 Nicoletti et al, 2008). This was followed by a progressive increase in vascularization. In both humans and rodents, the processes of angiogenisis and BBB disruption were still obvious in the chronic focus, probably the result of recurrent seizures (Marcon et al, 2009; Ndode-Ekane et al, 2010).

4.3 Inflammation, blood brain barrier and epilepsy

Several proinflammatory signals are rapidly induced in rodent brain during epileptic activity. These include cytokines, chemokines, prostaglandins, toll-like receptors, signal-transduction pathways that recruit nuclear factor-κB (NF-κB), complement factors and cell-adhesion molecules (Alapirtti et al, 2009; Avignone et al, 2008; Gorter et al, 2006; Manley et al, 2007; Natoli et al, 2000; Oliveira et al, 2008; Sinha et al, 2008; Zattoni et al, 2011). Seizures induce a massive inflammatory response in parenchymal cells, involving both microglia and neurons (Riazi et al, 2008, Zattoni et al, 2011).

Inflammation might either contribute to epileptogenesis or be a response that develops after seizures. There is substantial evidence supporting both CNS and intravascular inflammation as being seizure promoting or pro-epileptogenic. BBB damage is known to directly cause

seizures and to increase spontaneous seizure frequency (Rossi et al, 2011; Riazi et al, 2008; Vezzani et al, 2011,; Zattoni et al, 2011). Blockade of CNS or systemic inflammation pathways (e.g., via inhibition of interleukin [IL]-1β signaling with IL1-receptor antagonist or via blockade of IL-1β production with caspase-1 inhibitors) reduces status epilepticus and seizure frequency (Alapirtti et al, 2009; Gorter et al, 2006; Kim et al, 2010; Vezzani et al, 2010). Glia, neurons, and endothelial cells express cytokines following seizures in experimental models in human epileptogenic tissue and after brain injury (Rossi et al, 2011; Sinha et al, 2008). These findings point to a prominent role for cytokines in the pathogenesis of seizures. Elucidation of the mechanisms underlying the effects of cytokines in seizures highlights nonconventional modes of action involving direct effects on neuronal excitability or a direct action on BBB integrity.

Seizures also, however, induce elevated expression of vascular cell adhesion molecules and enhance leukocyte rolling and arrest in brain vessels, effects mediated by the leukocyte mucin P-selectin glycoprotein ligand-1 (PSGL-1, encoded by *Selplg*) and leukocyte integrins (Fabene et al, 2008; Gorter et al, 2006; Librizzi et al, 2007). Inhibition of leukocyte-vascular interactions, either with blocking antibodies, depletion of neutrophils or by genetically interfering with PSGL-1 function in mice, markedly reduced acute seizures and chronic spontaneous recurrent seizures (Fabene et al, 2008, Zattoni et al, 2011). Consistent with this experimental data there are also some clinical studies showing more abundant leukocytes in epileptic brains than in controls, pinpointing a potential leukocyte involvement (Zattoni et al, 2011). This, suggest that leukocyte-endothelial interaction could be a potential target for the prevention and treatment of epilepsy.

Taking into consideration all of these data, there is a need for the development of strategies to detect BBB permeability changes for diagnosis (i.e. to identify the epileptic region prior to surgery) and for targeting populations at risk of developing epilepsy. A diagnostic tool for measuring BBB permeability should give reliable, objective and quantitative information with high spatial resolution. Qualitative evaluation of BBB disruption in humans accompanied with analysis of the cerebrospinal fluid for serum proteins or peripheral blood for brain constituents (e.g. S100β) could be promising strategies (Marchi et al., 2003).

4.4 Blood Brain Barrier transporters and epilepsy

Changes in BBB transporter systems also play an important role in epilepsy. It is estimated that 20-25% of epileptic patients fail to achieve good control with the different antiepileptic drugs treatments, developing so-called refractory epilepsy. Changes in ABC transporters like P-gp, MRPs (MRP1 and MRP2) and BCRP are directly related with the refractoriness (Abbott et al, 2007; Dombrowski et al, 2001; Lazarowski et al, 2007; Liu et al, 2000; Löscher &Potschka, 2002). These transporters are overexpressed in the brains of patients with refractory epilepsy, with implications for active drug efflux from brain. The progressive neuronal P-gp expression, depending on intensity and time-constancy of seizure-injury, is in agreement with the development of "P-gp-positive seizure-axis" proposed by Kwan & Brodie, who also showed that the development of refractory epilepsy directly correlated with the number and frequency of seizures before initiation of drug therapy (Kwan & Brodie, 2005). Furthermore two recent studies highlighted a possible underlying mechanism of the increased Pgp protein expression during the seizures. The studies by Bauer and colleagues and Hartz and colleagues have indicated that NMDA receptor, cyclooxygenase-2 (COX-2) prostaglandin E2and NFκB are involved in increase expression of Pgp on brain

microvascular endothelial cells and subsequently with that specific COX-2 inhibitor, NMDA receptor antagonist and E2 receptor antagonist abolished seizers dependent increase in Pgp expression (Bauer et al, 2008; Hartz et al, 2006). Therefore, modulation of ABC efflux transporters at the BBB forms a novel strategy to enhance the penetration of drugs into the brain and may yield new therapeutic options for drug-resistant CNS diseases.

Another transporter prominent expressed in epilepsy is GLUT-1. GLUT-1 immunoreactivity is increased in blood vessels after status epilepticus and after kainic acid- or pentylenetetrazole-induced seizures (Cornford et al, 2000; Gronlund et al, 1996). As there is a rapid increase in neuronal metabolic energy demands during seizures (Gronlund et al, 1996), this indicates that GLUT-1 may be upregulated under conditions of elevated brain glucose metabolism. Alternatively, alteration in GLUT-1 expression may be relevant to angiogenesis, which contributes to epileptogenesis and/or ictogenesis in experimental and human epilepsy (Ndode-Ekane et al, 2010, Marcon et al, 2009).

5. Conclusion

The data from experimental animals and human clinical studies indicate that studying mechanisms underlying epileptogenesis and epileptic seizures must consider variety of interactions within the "neurovascular unit". Significant changes occur in the vascular system, astrocytes and microglia cells which contribute significantly to the pathogenesis of the disease. Recent advances in imaging indicate that identification and quantification of such events are in hand and call for large-scale prospective studies to explore the role of BBB breakdown in the epileptogenic process. Valuable information on the time resolution and extent of BBB permeability changes, the role of astrocytes, inflammation and specific molecular pathways in human epileptogenesis, may allow a better design of anti-epileptogenic and anti-epileptic treatments for specific populations.

6. References

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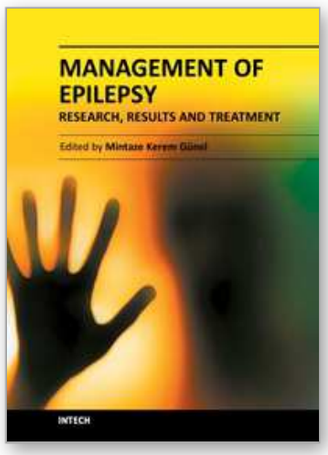

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Epilepsy is one of the most common neurological disorders, with a prevalence of 4-10/1000. The book contains the practical methods to approaching the classification and diagnosis of epilepsy, and provides information on management. Epilepsy is a comprehensive book which guides the reader through all aspects of epilepsy, both practical and academic, covering all aspects of diagnosis and management of children with epilepsy in a clear, concise, and practical fashion. The book is organized so that it can either be read cover to cover for a comprehensive tutorial or be kept desk side as a reference to the epilepsy. Each chapter introduces a number of related epilepsy and its diagnosis, treatment and co-morbidities supported by examples. Included chapters bring together valuable materials in the form of extended clinical knowledge from practice to clinic features.

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