Pharmacological Reports
2008, 60, 600–622
ISSN 1734-1140

Review

Physiology and pharmacological role of the blood-brain barrier

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Abstract:
The central nervous system (CNS) is a perfectly regulated environment with conditions far different from those in the rest of the organism. Even slight changes in this machinery affect its functioning. The blood-brain barrier (BBB) is the frontier that isolates brain tissues from the substances circulating in the blood vascular system. It is also a diffusion barrier that allows only water and small lipophilic molecules to freely access the brain in accordance with their concentration gradients. Moreover, animal studies have revealed differences in the barrier tightening time-course during development. The BBB becomes resistant to larger molecules before it stops smaller ones. Thus, its maturation has a progressive scheme. A similar scheme is true for BBB transporters. Due to all of these facts, the BBB is the most significant element responsible for the preservation of CNS homeostasis.
As a functional system, the BBB can be investigated as a frontier composed of pericytes, astrocytic end feet, and brain endothelial cells (ECs). Special emphasis is placed on the tight junctions (TJs) existing between them. An alternative point of view considers the BBB to be a functional complex consisting not only of bricks of cells but also of structures between those cells and their co-functioning elements.

Key words:
blood-brain barrier, endothelium, tight junctions, transport systems

Introduction

For optimal activity, the central nervous system (CNS) requires a perfectly regulated environment and homeostasis with characteristics far different from those in the rest of the organism. The main factor maintaining the homeostasis of the CNS is the proper function of the blood-brain barrier (BBB). Under both physiological and pathological conditions, the BBB isolates and protects nervous tissue of the brain and spinal cord from fluctuations in nutrients, hormones, metabolites, and other blood constituents. It also protects this tissue from the direct influences of many endo- or exogenous compounds circulating in the

blood. The BBB is necessary and especially important for the highly precise control of the microenvironment that secures neuronal transmission [7, 36, 81, 83].

The term blood-brain barrier actually encompasses a few aspects of barrier functions; these include physical, transport, and metabolic aspects. Briefly, the BBB tasks of great importance for CNS functions can be divided into a few categories. The BBB controls delivering of nutrients to and removing metabolites from the CNS compartment. It is also a barrier for ionic currents and hydrophilic compounds able to cross it only through special membrane transporters. The BBB protects the CNS from abrupt changes in blood biochemistry that occur after meals, physical excercises, or in pathological conditions. Moreover, the BBB divides neurotransmitters and in general, neuroactive substances into two pools – of the central and peripheral systems [7].

The BBB also plays a key role in CNS disorders, in which barrier permeability usually increases significantly. Moreover, the BBB is also an extremely important „guardian" that regulates access of drugs to the CNS under both physiological and pathological circumstances. Only a small number of drugs are able to freely penetrate the BBB and gain access to nervous tissue. BBB components are responsible for the biochemical modification of chemicals that enter the CNS. Moreover, some drugs, even if they can cross the BBB, are immediately removed from the CNS.

To ensure and protect CNS homeostasis, the BBB has to be a very stable structure. However, for fast adaptation to changing conditions, the BBB also requires plasticity [41]. This characteristic depends on many structural and functional properties of BBB components.

BBB building blocks

Endothelial cells

Brain endothelial cells (ECs; Fig. 1) are unlike the endothelial cells of the peripheral circulation. In comparison to those cells, brain ECs, differ phenotypically, are characterized by the presence of tight junctions (TJs), and lack pinocytic vacuoles. The high number of cytosolic mitochondria suggest their high energy metabolism. It is also noteworthy that they lack fenestrations in their plasma membranes [2, 21, 41, 58] and are selectively permeable to molecules with suitable mass and lipophilicity [41].

ECs have several specific identity markers, including γ-glutamyl-transpeptidase (GGTP), alcalic-phosphatase, von Willebrand factor (vWf), and glucose transporter-1 (GLUT-1). Endothelial barrier antigen (EBA) and OX-47 antigen merit mention here as well [21]. The luminal surface of endothelial cells is covered by glycocalyx – negatively charged mesh of proteoglycans, glycosaminoglycans, glycoproteins and glycolipids [219]. Furthermore, BBB ECs are also marked by the presence of two N-glycosylated phosphoproteins: P-glycoprotein (Pgp) and Multidrug Resistance-Associated Protein (MRP) [20]. Finally, their most important feature is their tight junctions.

Astrocytes

Astrocytes, like neurons, derive from the ectoderm of the neural tube. Of the approximately 11 phenotypes ascribed to astrocytes, eight correlate with blood vessels [6]. Furthermore, their cross-cellular interactions enforce functional polarity [3, 174]. Two main types of astrocyte cells can be distinguished in the brain. Protoplasmic cells exist in the grey matter, and fibrillary cells are present in the white matter. Protoplasmic astrocytes are characterized by large nuclei and numerous thick cytoplasmic appendices. The endings of the appendices form cap-like structures known as end-feet (lat.: *pes sugens*) that tightly attach to neurons on one side and blood vessels on the other to form a specific relay station between neurons and blood.

Due to their localization, astrocytic end feet have a few specific characteristics. For example, high concentrations of the water channel aquaporin 4 (AQP4) and the K^+ channel Kir4.1 are placed within the orthogonal arrays of particles (OAPs). This accumula-

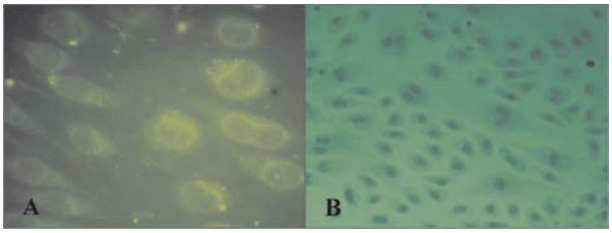

Fig. 1. Rat brain capillary endothelial cells-*in vitro* culture. **(A)** Immunofluorescent visualization of VWF; **(B)** cells stained with Hematoxylin-Eosin

tion is connected with the expression and action of agrin – heparin sulphate proteoglycan. This protein is produced in the extracellular matrix sheet by the basal cell membranes (basal lamina) [221, 231]. Agrin is important for BBB integrity and accumulates when the barrier tightens [231]. Furthermore, its splice variant Y0Z0 is a specific element of the capillary basal lamina formed by brain ECs. The above-mentioned specific details suggest a vital role for the extracellular matrix in the cross-influence of astrocytes and endothelium [6].

Astrocytes play a major role in neuronal metabolism, nutrition, and discharge of used substrates. Astrocytes that connect to the pial matter have the abilities of transcytosis and active ion transport.

Pericytes

Pericytes are small vessel wall-associated cells that originate developmentally from the mesoderm and differ from mesenchymal cells [105]. They are separated from ECs by the basal lamina (basement membrane), but gap junctions (peg-and-socket) provide contact spots [50].

In the rat brain, pericytes cover approximately ¼ of capillary outer surfaces [194]. There are two classes of pericytes: some are placed at capillary straight parts, whereas others are located at capillary connections. Anatomically, they are equipped with claw-like appendices entangling the capillary [162]. Co-culture studies have revealed that capillaries change their phenotype from multilateral to spindle-like when they connect to capillary-like structures (CLS); however, this type of association occurs only in the presence of astrocytes [169].

In the brain, pericytes are responsible for the regulation of EC activity, mediation of inflammation, and control of CLS formation and capillary diameter [22, 140, 162, 169]. They therefore play an important and composite role in the maintenance of the BBB and brain homeostasis.

Tight junctions (TJs)

The most important factors responsible for BBB impermeability are the junctional complexes existing between the ECs of brain microvessels. TJs exist between the ECs and encircle the cells like a continuous belt. Morphologically, they are represented by closely connected fragments of adjacent ECs known as the zonula occludens. At these locations, membranes are completely fused and form a five-layer construction [21]. The number of fusion points between TJs differs; due to these differences, the level of tightness in different regions is also diverse [21].

Functionally, TJs work in several ways. They constitute the frontier for protein and lipid diffusion across the membranes and confer to the ECs polarity, which is manifested by a non-uniform distribution of a number of transporters between the luminal and abluminal membranes. Due to complete fusion, they also seal the paracellular way to force transport of substances through the membranes and cytosol [21, 76].

TJs are characterized by high electrical resistance (1500–2000 Ωcm^2), and their integrity depends on a proper extracellular Ca^{2+} ion concentration. A drop of resistance causes TJ destabilization. Moreover, an increase in intracellular cATP causes the formation of fusion points and tightens TJs. Opposing mechanisms seem to be mediated by phorbol esters.

Several plasma membrane proteins forming TJs have been identified. Among those are claudin, occludin, and adherens junction molecules. In addition, ZO (zonula occludens)-1, ZO-2, ZO-3, and cingulin have been identified as cytoplasmic proteins that link transmembrane proteins with actin, a primary cytoskeletal protein responsible for the structural and functional integrity of the endothelium [21].

Claudins

To date, 24 members of this protein family have been identified in mice and humans. Claudins are 22 kDa phosphoproteins consisting of four transmembrane domains and seem to be the primary building material of the TJs. The claudin from one EC connects with an analogous claudin from an adjacent EC to create the "primary closure" of the TJ, and the carboxylic end of each protein links it to cytoplasmic ZO-1, ZO-2, or ZO-3.

In brain tissue, claudin 1/3, 5, and 12 have been identified [6]. Along with occludin, claudin 1/3 and 5 are present in the endothelium. Claudin-5 has been shown to be expressed particularly in brain capillary endothelial cells (BCECs) [195]. Immunocytochemical analysis of the human cerebral cortex revealed

similarities in expression pattern between claudin-5, occludin, and JAM-1 [224].

Occludin

Occludin is a phosphoprotein larger than claudin (60 kDa), but it also consists of four transmembrane domains [18]. The two extracellular loops of claudin and occludin form the paracellular component of the TJ, and the cytoplasmic domain is tightly connected with ZO proteins.

Occludin expression has been found in rodents and humans, but not human newborns or fetuses. Recent experiments have shown that occludin expression is highest among those of all junctional proteins. Immunocytochemical studies revealed numerous signals for occludin localized either to membranes of adjacent ECs or to the crevasse between them [224].

It seems that occludin function is regulatory and may influence paracellular transport [86]. Occludins and claudins form heteropolymers and transcellular tracts containing channels for the selective transport of ions and hydrophilic molecules [131]. Furthermore, occludin may be involved in maintaining BBB electrical resistance and aqueous pore formation [218]. These facts, together with the relationships existing between occludin and claudin, suggest the contribution of these proteins to the selectivity of TJ-associated diffusion [163]. Their presence seems to be essential for the proper function of TJs and the BBB.

Junctional adhesion molecules (JAMs)

These molecules belong to third group of membrane proteins involved in TJ construction. They are immunoglobulins with molecular masses around 40 kDa. Their single transmembrane domain is linked with an extracellular fragment consisting of two "immunoglobulin-like" loops [163].

To date, three members of this family (JAM-1, JAM-2, and JAM-3) have been identified in the rodent brain. In addition, JAM-1 and JAM-3 are localized to brain vessels. Recently, Vorbrodt et al. [224] found JAM-1 in the human cerebral cortex. Immunocytochemical analysis exposed signals representing JAM-1 distributed unevenly in interendothelial junctions, where it was found both alone and forming small clusters.

JAMs may play a role in cell adhesion and monocyte migration through the BBB [159].

Cytoplasmic proteins

Cytoplasmic proteins implicated in TJ construction include zonula occludens proteins (ZO-1, ZO-2, and ZO-3), cingulin, and 7H6.

ZO proteins are membrane-associated guanyl kinase-like proteins (MAGUKs). They are composed of three PDZ domains (PDZ1, PDZ2, and PDZ3), one SH3 domain, and one guanyl kinase-like domain (GUK). These domains are important because of their role in protein arrangements, and consequently in plasma membrane integrity. Immunosignals of ZO-1 were frequently seen as complexes in short fragments of the junction with long unlabeled intervals [224].

It was shown that the PDZ1 domain of ZO proteins binds directly to the carboxylic end of claudin [95], whereas occludin interacts with the GUK domain in ZO-1 [136]. Furthermore, JAM proteins bind directly to ZO-1 and other PDZ-containing proteins [63], with actin connected to the carboxylic ends of ZO-1 and ZO-2 [82]. In addition, immunocytochemical observations by Vorbrodt et al. [224] revealed a close structural relationship between immunolabeled occludin and ZO-1 [224].

Another junction-associated protein is 155 kDa 7H6, a phosphoprotein correlated with TJ impermeability to ions and large molecules [182]. In contrast to ZO-1, 7H6 may detach from the TJ when ATP levels fall; this detachment results in increased paracellular permeability. It therefore maintains its relationship with the functional state of the junction [135].

Cingulin is a 140–160 kDa phosphoprotein localized at the cytoplasmic side of TJs. It has been shown that cingulin binds to ZO proteins, myosin, and AF-6, and this binding implies its role as a scaffold between transmembrane proteins and the cytoskeleton [45].

Other cytoplasmic proteins with adaptor, regulatory, and signaling functions are: Ca^{2+}-dependent serine protein kinase (CASK), partitioning defective proteins (PAR3 and 6), multi-PDZ-protein 1 (MUPP1), membrane-associated guanylate kinase with inverted orientation of protein-protein interaction domains (MAGI-1, -2, and -3), small GTPases, G-protein signaling 5 (RGS5), and ZO-1-associated nucleic acid-binding protein (ZONAB). The recently discovered junction-associated coiled-coil protein (JACOP) may take part in binding the complex to the cytoskeleton [6].

On the basis of the above remarks, an assumption can be made regarding the role of TJ proteins. It seems that they not only maintain the integrity of the

junction but also provide structural support for the brain endothelium due to the multiple connections between them. Disturbances in this arrangement influence cell structure as well as TJ integrity. Consequently, BBB functions and CNS homeostasis will be altered.

Adherens junctions (AJs)

Adherens junctions provide a second example of tightening structures between ECs in which transmembrane proteins are represented by the large family of cadherins. Cadherins interact with each other homotypically when calcium ions are present [206]. In AJs, catenins play a role analogous to that of ZOs in TJs by anchoring cadherins to the actin cytoskeleton. It is believed that β- and γ-catenin mediate cadherin connection to α-catenin, which in turn links the whole complex to the cytoskeleton [144]. Interestingly, recent experiments revealed the presence of a new catenin in the ECs. p120 Catenin binds preferentially with VE-cadherin (vascular endothelial cadherin), but the significance of this discovery to BBB functioning requires further studies [11].

Studies have revealed that VE-cadherin and catenins are present in the human cortex. Numerous immunosignals for VE-cadherin were observed by Vorbordt et al. [224]. They were scattered along the junction, and the majority were located in or close to the cleft. Conversely, immunosignals representing catenins were much more random and localized further from the cleft (α-, β-, and p120 catenin) or in its close proximity (plakoglobin) [224].

In addition, studies have shown that human AJs express platelet endothelial cell adhesion molecule-1 (PECAM-1), which takes part in shaping AJs *via* interactions with β-catenin [130].

The proximal spatial organization of proteins comprising AJs and TJs indicates that both junction types contribute to the human BBB [224]. Moreover, evidence exists that ZO-1 and catenin cooperate, which suggests that TJs and AJs work in concert [131]. Nevertheless, an observation-based hypothesis suggests that cadherins and catenins anchor ECs to pericytes or smooth muscle cells during the development and maturation of the BBB in certain species (e.g., chicken). This hypothesis therefore implicates these proteins in roles different from those they play in human-type BBB microvessels [224]. These findings suggest that species-related differences exist between components of BBB junctions.

Transporters

The physiological function of the BBB is essential in the CNS. For instance, it is important for both the inactivation and reuptake of neurotransmitters after their pre-synaptic secretion. Furthermore, one of the major functions of the BBB is the regulation of the transport of nutrients and other molecules into and out of the brain. Thanks to TJs, only lipid-soluble substances and substances possessing a transportation system can cross the BBB [37, 152]. Taking all of these facts into account, the importance of understanding BBB functions (especially in light of various CNS disorders) seems obvious [149].

In addition to their other functions, tight junctions between brain ECs divide the membranes of ECs into two sides: a blood-facing luminal membrane and a brain-facing abluminal membrane. This division also accounts for proteins present in each part of the cell membrane, and joint distinctiveness of these membranes determines if and how quickly particles traverse the BBB [83]. In 1985, the supposition was made that the blood-brain influx system is of key importance for the permeability of CNS-acting drugs and nutrients. Because of this, molecular BBB transport has been analyzed mainly in this aspect. Subsequently, amino acid transporters were discovered [49]. When it was more recently found that P-gp (poly-glycoprotein) takes part in xenobiotic efflux [187, 216], the BBB transport system began to be regarded as a far more complex system [147].

Recently, a novel method for quantitative focused proteomics has been developed. This state-of-the-art technique allowed for the construction of a quantitative atlas of brain capillary endothelial cell membrane transporters (Mdr1a, Mrp4, Bcrp, 4F2hc, Asct2, Glut1, Mct1, Lat1, Oat3, Oatp2, Oatpf, and Taut). Both existing data and further usage of the technique may provide great insight into the complex physiology and pharmacology of the BBB transportation systems [102].

The barrier and ATP-binding cassette (ABC) transporter superfamily

ATP-binding cassette transporters comprise one of the largest protein families, and they are crucial for a number of biomedical aspects like drug transport and resistance to cancer and xenobiotics [31]. These transporters are membrane proteins consisting of many domains that use ATP-bound energy for the transport of solutes across the cell membrane in all mammals [98]. Members of this family include MDRs (Pgp), MRPs (ABCC family), and BCRP (Fig. 2).

Multidrug resistance proteins (MDRs)

MDRs were primarily recognized in mammalian tumor cells, where they confer multidrug resistance [77]. Their encoded protein product is P-glycoprotein (Pgp), a 170 kDa transporter for a broad variety of substrates [123]. It was the first ABC transporter localized in the human BBB [46, 213]. Subsequent detection in numerous other mammals (e.g., monkeys, rats, mice, and pigs) suggested its importance as a BBB defense system against supposedly dangerous lipophilic endo- and xenobiotics [184].

Two known MDRs (Pgps) exist in humans. MDR1 Pgp is encoded in humans by the *MDR1* and in rodents by the *Mdr1a* and *Mdr1b* genes. MDR1 Pgp is involved in the exertion of numerous amphipathic and hydrophobic compounds (including drugs) from tumor cells. Under normal conditions, it functions as an efflux pump existing in endo- and xenobiotic-eliminating organs (e.g., the kidney and liver), as well as tissues responsible for protection from xenobiotic entry (e.g., the small intestine, testes, placenta, and BBB) [77, 123, 185]. Interestingly, experiments revealed far more abundant expression of Mdr1a in the BBB than in the liver or kidney [102].

MDR2 Pgp, which is encoded by the *MDR2* and corresponding *Mdr2* genes, is present in the canalicular membranes of hepatocytes and functions as a phosphatidylcholine translocase. It does not influence multidrug resistance [55, 77, 185].

Mdr1 Pgp expression has been shown in mice brain capillary endothelial cells (BCECs) [187], where it immunohistochemically localizes in the luminal membranes [25, 55, 73, 186, 201] (Fig. 2). Therefore, its substrates that enter the EC are immediately secreted

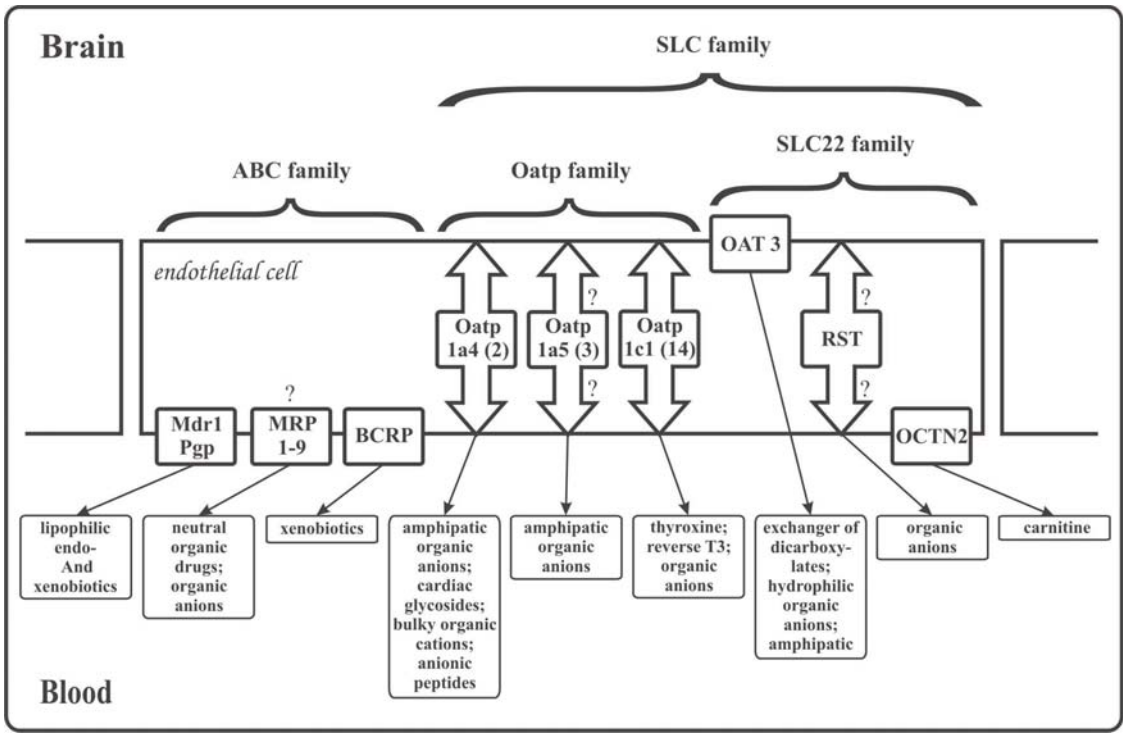

Fig. 2. Membrane localization and substrates of the BBB transportation systems. Explanations are in the text

back into the blood [123]. Its protective function has been shown in studies in which *Mdr1a*-deficient mice expressed much higher sensitivity to a neurotoxin (ivermectin) than wild type animals [17]. Those results underline the importance of *Mdr1a* in protection against toxic compounds in the CNS.

Experimental data have also suggested the existence of a CNS-targeted drug delivery pathway occurring through co-administration with Pgp inhibitors. Generally, more lipid-soluble substances more easily penetrate brain tissue. Many lipid soluble drugs useful in therapy are substrates for ABC efflux transporters, however, and their uptake by brain is thus inhibited. Studies show that the brain penetration of drugs that are Pgp substrates can be increased in *Mdr1* knock-out mice up to 100-fold, although such penetration has consequences for the toxicity of the compounds [186]. Furthermore, *Mdr1a*-deficient mice exhibited significantly higher penetration of ivermectin and a number of other compounds (e.g., HIV protease inhibitors, immune suppressants, digoxin, dexamethasone, and anticancer drugs) [17]. Similarly, an increase in drug brain penetration was observed with the use of Pgp inhibitors as well [31, 124].

Recently, it has been shown that Pgp is expressed dually in brain [223]. Under normal physiological conditions, Pgp is primarily expressed by ECs as well as parenchymal and perivascular astrocytes. Pathological states (seizures) upregulate Pgp expression in these other cell types and increase its *de novo* synthesis in neurons [222, 223]. These data suggest a more complex role for Pgp than previously supposed.

Recent experiments had shown genetic polymorphism of intestine-derived Pgp, and further data support this assumption in other organs [70, 87]. There is a chance that such a polymorphism confers differences in BBB Pgp functioning between individuals. Nevertheless, whether genetic polymorphisms account for brain-derived Pgp as well as Pgp's association with specific functions remain to be revealed.

MDR-associated proteins (MRPs, ABCC family)

To date, the ABCC family of transporters consists of 12 members including MRP1-9 (Fig. 2). Their expression at the BBB has been confirmed for several animal species. MRP1 and MRPs4-6 have been detected in primary cultured bovine brain microvessel ECs and the capillary-enriched fraction from bovine brain homogenates [236] along with MRP2 at the luminal ECs membranes of isolated capillaries from rat and pig brain [134]. Furthermore, cDNA array analysis revealed high MRP1 mRNA levels and low levels of MRP2, MRP3, and MRP5 mRNAs in human BCECs. While these transporters are able to transport neutral organic drugs, they primarily transport organic anions [32]. Nevertheless, it has to be emphasized that the subcellular localization of most of these transporters remains to be elucidated or confirmed (MRP2). Only luminal localization would be relevant in the restriction of substrate brain penetration [123].

The best characterized members of this family of transporters are MRP1 and 2. MRP1 is a primary leukotriene C4 transporter. It confers protection against toxic compounds; when overexpressed, it also mediates resistance to numerous antitumor agents (e.g., vincristine and daunorubicin) [170].

MRP2, also a canalicular multispecific organic anion transporter (cMOAT), is expressed in the kidney, liver, and small intestine. Studies show that it is a significant part of hepatocytic detoxification system, where it removes anionic glucuronides along with endo- and xenobiotic-derived glutathione conjugates and unconjugated organic anions into the bile [113].

Experiments with MRP inhibitors (e.g., probenecid, MK-571) have indicated their role in BBB permeability. After inhibitor application, either drug infiltration into the brain is enhanced or drug efflux from isolated EC is inhibited [124]. Furthermore, other experiments have revealed that MRP2 expression restricts the transport of the anti-epileptic drug phenytoin through the BBB.

Recently, the role of MRP4 has been investigated in *Mrp4* knock-out mice [120]. The results of this study exhibited the dual characteristics of this transporter's expression. MRP4 was expressed at the luminal side of brain capillaries, whereas it was expressed at the basolateral membrane in the choroid plexus. Furthermore, it has been shown that MRP4 limits the blood-to-brain and blood-to-CSF transport of substrate drugs (e.g., topotecan). The authors of this work suggested that MRP4 acts to inhibit the brain penetration of toxic anionic compounds as well as therapeutic organic anions and transports toxic metabolites (e.g., 1-napthol glucuronide) from the brain.

Breast cancer resistance protein (BCRP)

Another protein that seems to be important in BBB transport and permeability is BCRP (Fig. 2). Its simi-

larities with Pgp's localization in tissues suggest its possible role in the protection from xenobiotics [186]. It was primarily discovered in a chemotherapy-resistant breast cancer cell line. Recent experiments revealed its presence at the luminal membrane of pig, mouse, and human BCECs. Moreover, BCRP expression in BBB cells was stronger than that of MRP1 or Pgp. Experiments with *mdr1a* knock-out mice showed upregulation of BCRP in brain capillaries in response to the lack of Pgp. In addition, BCRP inhibition resulted in reduced penetration of prazosine and mitoxantrone.

Recent experiments with *Bcrp* knock-out mice revealed that BCRP limits the availability as well as brain and male reproductive system penetration of phytoestrogens (e.g., daidzein and genistein) after their oral administration. BCRP also extruded these compounds from the placenta to the blood. These result show BCRP's protective role against unfavorable effects of plant-derived estrogen-like compounds [64].

How ABC transporters distinguish and translocate their substrates still remains to be elucidated [5]. In addition, the overlap between their substrates also has to be explained.

The barrier and the solute carrier family (SLC family)

The SLC family of transporters is an important part of the organic anion efflux system. It consists of organic anion transporting polypeptides (OATP/*SLCO*) and organic anion transporters (OAT/*SLC22A*). They cooperate with MDRs and MRPs in the removal of xenobiotics from the brain.

A total of 300 genes belonging to the superfamily have been divided into 43 families, and the SLCO/SLC21 and SLC22A families demonstrate multispecificity and play a role in the BBB efflux transport system of organic compounds (Fig. 2).

SLCO/SLC21 (Oatp/OATP) family

Oatp/OATPs are expressed in numerous tissues like the choroid plexus, lung, heart, intestine, kidney, placenta, testis, and BBB. However, some of them are selectively expressed in the liver and play a role in endo- and xenobiotic elimination [207]. Numerous amphipathic organic solutes (e.g., bile salts, organic dyes, steroid conjugates, thyroid hormones, neuroactive peptides, and various drugs) are substrates for Oatp/OATPs, and many of these are polyspecific organic anion transporters [132]. Of the fourteen members of this family, expression of Oatp1a4 (Oatp2), Oatp1a5 (Oatp3), and Oatp1c1 (Oatp14) has been shown in brain microvessels (Fig. 2).

Studies revealed abundant Oatp1a4 expression in the brain [7, 145], where it is localized at the luminal and abluminal membranes of the capillaries as well as on the basolateral membrane of the choroid plexus ECs [73]. Its substrate specificity is broad and includes amphipathic organic anions (bile acids, steroid conjugates), cardiac glycosides (digoxin, ouabain), bulky organic cations (N-(4,4-azo-n-pentyl)-21-deoxyajmalinium, N-methylquinidine, N-methyl-quinine and rocuronium), and anionic peptides (BQ-123, [D-Pen2,D-Pen5]-enkephalin, deltorphin II) [7, 72, 145, 173, 220]. Asaba et al. [16] reported that the Oatp1a4 (oatp2) transporter is involved in dehydroepiandrosterone (DHEAS) elimination through the BBB [16]. Moreover, membrane transporter localization [73] supports the theory that BBB-expressed Oatp1a4 (oatp2) plays a role in DHEAS efflux transport. Interestingly, studies also suggest that is involved in uptake process of [D-penicillamine 2,5]-enkephalin and taurocholate [51].

Oatp1a5 expression has been observed in the brain, small intestine, lung, retina [226], BBB, and choroid plexus [116, 150]. Oatp1a5 is responsible for the uptake of amphipathic organic anions, including bile acids, steroid conjugates, and thyroid hormones, from the CSF [7, 40, 116, 226]. Although its association with brain capillaries has been shown by immunofluorescence, its exact membrane localization remains to be determined [150].

The only human ortholog, OATP1A2, is known to be expressed in the lung, liver, kidney, and testis as well as more abundantly in the brain [8]. Although its membrane localization remains unknown, more precise immunofluorescence studies have suggested its localization in BCECs [71]. Known substrates include amphipathic organic anions, type II organic cations, and peptides ([D-penicillamine 2,5]-enkephalin, deltorphin II) [33, 72, 115].

Apart from DHEAS, it has been shown that Oatp1a4 (Oatp2) and OATP1A2 (OATP-A) are involved in the transport of other steroids (estrone-3-sulfate, estradiol-17B-glucuronide), thyroid hormones (T_3 and T_4), drugs (fexofenadine), cationic compounds (rocuronium,

ADP-ajmalinium), and peptides exerting neuroactivity ([D-penicillamine 2,5]-enkephalin) [51, 77, 199]. On the basis of those facts, a supposition can be made that BBB-localized Oatp1a4 (Oatp2) and OATP1A2 (OATP-A) work as either 1) efflux pumps for both substrates synthesized in the brain and active in the margin and waste products designated for excretion or 2) influx pumps for neuroactive compounds like opioid peptides [77].

Oatp1c1 was originally described as the BBB-specific anion transporter 1 (BSAT1) [122]. Studies revealed its luminal and abluminal membrane localization in rat and mouse brain capillaries as well as at the basolateral membrane of the mouse choroid plexus [200, 214]. Among its substrates, the highest transport activities have been demonstrated for thyroxine and reverse T3. In addition, it is able to transport organic anions (e.g., E217βG, cerivastatin, and troglitazone sulfate) [200]. Similarly to Oatp1c1, the human ortholog, OATP1C1 (*SLCO1C1*) has the highest transport efficiency for thyroxine and reverse T3. Furthermore, it is also widely expressed in the brain (except for the cerebellum) as well as testis Leydig cells [166].

SLC22 family

To this family belong organic cation transporters (Oct/OCT, *SLC22A1-3*), organic cation/carnitine transporters (Octn/OCTN, *SLC22A4, -5*), organic anion transporters (Oat/OAT, *SLC22A6-8, -10, -11*), and related transporters (e.g., URAT1/renal specific transporter (RST) (*SLC22A12*) and CT2 (*SLC22A16*). BBB-expressed members include OAT3, OCTN2, and RST [65, 66] (Fig. 2).

Oat3 is a homolog of Oat1 expressed in the liver, kidney, brain, and eye [117], but the molecular mass of the protein expressed in brain is greater than that in the kidney due to differences in its glycosylation state. Immunochemical analysis of rat brain capillaries revealed abluminal localization of the Oat3; but weak signals were also visible at the luminal side [107]. Moreover, mRNA for Oat3 was also detected in immortalized rat BCECs [146]. In the human choroid plexus, OAT1 and OAT3 expression has been shown; however, their membrane localizations remains to be determined [10].

Oat3 substrates include amphipathic organic anions (E217βG, estrone sulfate, DHEAS), hydrophilic organic anions (PAH, benzylpenicillin), and organic cations (cimetidine, ranitidine) [117, 141, 142]. In addition, it has been suggested that Oat3/OAT3 is an exchanger driven by an outward concentration gradient of dicarboxylates [117, 202].

Evidences indicate that rat and mouse Oat3 can transport homovanillic acid (HVA), the main dopamine metabolite [138, 148]. The localization supports the hypothesis of its role in brain-blood HVA efflux transport. In addition, many anionic metabolites of neurotransmitters (but not neurotransmitters themselves) inhibit HVA transport by Oat3 [138]. This indicates the possibility of Oat3 involvement in the efflux transport of various neurotransmitter metabolites. However, it has to be emphasized that an inhibitor does not necessarily need to be a substrate.

Renal-specific transporter (RST) is a mouse homolog of the urate transporter. Its expression has been predominantly shown in the kidney. Further experiments led to its detection in the brain capillary-enriched fraction and choroid plexus, but its membrane localization remains unknown [93]. It has been supposed that RST is a facilitative transporter able to mediate efflux [93] and coordinate Oat3 in organic anions transportation at the BBB and BCSFB (brain-cerebrospinal fluid barrier).

Octn2/OCTN2 is characterized as a sodium-dependent carnitine transporter [191, 209] capable of tetaraethylammonium (TEA) transfer [208]. Three isoforms have been described in rodents (Octn1-3) and two in humans (OCTN1 and OCTN2). The expression of Octn2 has been shown in primary cultured rat BCECs as well as the whole brain [106]. Studies revealed that loss of Octn2 causes a decrease in the brain acetyl-carnitine concentration, suggesting its luminal localization at the BBB [94]. On the basis of experimental results, a hypothesis of its involvement in the efflux of organic cations in exchange for plasma carnitine or acetyl-carnitine has been developed [94, 106, 143, 146, 234].

Experimental data regarding the presence of the members of the Oct/OCT transporter family at the choroid plexus exist [99, 100, 203, 237], but further studies are needed to elucidate their presence and function at the BBB.

The barrier and amino acid transport

Polar organization accounts for transport protein localization in ECs and mediates amino acid homeosta-

sis in the brain. At least three Na^+-dependent EAATs (excitatory amino acid transporters) and one Na^+-dependent system transporting glutamine exist at the abluminal side, whereas facilitative transporters for glutamate and glutamine are present only on the luminal side. Such organization restricts glutamate penetration to the brain and supports acidic and nitrogen-rich amino acid removal from the CNS. In addition, there are two facilitative neutral amino acid (NAAs) transporters present at both membranes of ECs. These carriers ensure the delivery of vital amino acids to the brain. Finally, four Na^+-dependent NAAs transporters at the abluminal side cooperate to keep NAA concentrations in the brain ECF at levels roughly1/10 of the plasma concentration [83].

Large neutral amino acid facilitative transport (L1 system)

The influx of essential amino acids to the brain is greater than the movement of nonessential amino acids [23, 154] and occurs *via* facilitative and Na^+-independent transport. The transporter responsible seems to be the L1 system (Tab. 1) [21, 30, 109, 190]. While this system is present in both membranes, it is twice as abundant at the luminal side.

Cationic amino acid facilitative transport (y^+ system)

The y^+ system is comprised of an amino acid transporter with affinity for amino acids containing cationic side chains (Tab. 1). Interestingly, it shows a frail interaction with NAAs in the presence of Na^+ [125, 229]. The y^+ is system present on both sides and is more abundant at the abluminal membrane.

Facilitative glutamine transport (n system)

The BBB glycine transport system has been described by Lee et al. [119] not to demonstrate trans-stimulation, and it is similar to that existing in hepatic plasma membrane vesicles [156]. It exists only at the luminal membrane [119] and is inhibited by aspargine and histidine.

Facilitative transport of acidic amino acids (x_G- system)

It has been suggested that glutamate transport is of a facilitative nature and is mediated by the x_G- transporter [26]. Those data are supported by the results of Lee et al. [119], who found facilitative transport only at the luminal side that mediating transport from the ECs to the plasma.

The barrier and Na^+-dependent amino acid transport

Na^+-dependent systems include the A system (alanine-preferring), ASC system (alanine-, serine- and cyste-

Tab. 1. Amino acid transporters localized at the endothelial cells of the BBB

Transporter	Na^+-dependent/facilitative	Membrane		Substrates
		Luminal	Abluminal	
L1	–/+	++[1]	+	Asn, Gln, Leu, Val, Met, His, Ile, Tyr, Trp, Phe, Thr
y^+	–[2]/+	+	++	Lys, Arg, Orn, homoarginine
x_G-	–/+	+	–	Asp, Glu
n	–/+	+	–	Asn, Gln, His
Na^+-LNNA	+/–	–	+	Gly, Ala, His, Thr, Met, Val, Leu, Ile, Phe, Tyr, Trp
A	+/–	–	+	Ala, Ser, Pro, Asn, Gln
ASC	+/–	–	+	Gly, Ala, Ser, Thr, Met, Val, Leu, Ile, Phe, Tyr, Trp
N	+/–	–	+	Ser, Asn, Gln, His
EAAT	+/–	–	+	Asp, Glu

[1] Number of pluses corresponds to abundance. [2] Shows a frail interactions with NAAs in presence of Na^+

ine-preferring) [80, 211, 215], N system (glutamine-, asparagine- and histidine-preferring) [119], excitatory amino acid transporter (EAAT) family (aspartate- and glutamate-preferring) [90, 152], and Na^+-LNAA (essential large neutral amino acid) system. Evidence suggests that only facilitative transport occurs at the luminal membrane [42, 154, 192]. Therefore, Na^+-dependent transporters are present only at the abluminal membranes. Using the Na^+ gradient between ECF and ECs, they are responsible for the removal of AAs from the CNS.

Na^+-dependent large neutral amino acid transport (Na^+-LNAA system)

The Na^+-LNAA system is a Na^+-dependent, BCH-inhibited transporter of large neutral amino acids (LNAAs) that is localized at the abluminal membrane [151]. Its kinetics differ from those of any other known transporter. In addition to Na^+-dependency and BCH-inhibition, it is also voltage sensitive. Na^+-LNAA substrate specificity is similar to that of L1 and allows gradient-oriented entry of essential LNAAs (Tab. 1). Those facts suggest its involvement in the control of the LNAA brain content [83].

Na^+-dependent small nonessential neutral amino acid transport (A system)

The A system differs from other Na^+-dependent transporters by acceptance of N-methylamino-isobutyric acid (MeAIB) as a substrate [43]. It is voltage sensitive [153], prefers alanine [155], and is inhibited by proline, histidine, alanine, serine, asparagine, and glutamine.

The hypothesis of A system presence at the abluminal membrane of the BBB is supported by the results of experiments on isolated rat brain capillaries and isolated abluminal membrane vesicles from bovine BCECs [28, 180]. Expression of all three isoforms of system A (ATA1, ATA2, and ATA3) has been seen in the rat brain capillary rich fraction and conditionally immortalized rat BCECs (TR-BBB) cells, ATA2 showed the greatest expression. This result suggests that this isoform exactly is responsible for system A transport at the BBB [147]. Under hypertonic conditions, ATA2 transcription is up-regulated. This finding suggests that the BBB system A is regulated by the osmolarity. Thus, ATA2 osmolarity regulation may play a role in maintaining proper BBB functioning under pathological conditions [147].

Na^+-dependent large and small neutral amino acid transport (ASC system)

The presence of this system in abluminal membranes was confirmed after system A blockade with MeAIB [80, 211, 215]. The ASC system seems to be voltage independent with wide substrate specificity (Tab. 1) [153].

Na^+-dependent nitrogen rich amino acid transport (N system)

This system is voltage independent [153]. The possibility of Na^+ replacement with Li^+ suggests its resemblance to the N system existing in the liver [108, 153]. Transported AAs are listed in Table 1.

Acidic amino acid transport (EAAT family)

It is believed that the BBB might be the control site for the maintenance of nontoxic glutamate levels. Experiments show at least three Na^+-dependent glutamate transporters existing within the BBB. When combined, these transporters demonstrate high glutamate affinity. O'Kane et al. [152] have shown the presence of EAAT1, 2, and 3 protein in bovine brain endothelial cells. Moreover, they have shown that each of these transporters is present at the abluminal membrane of ECs, voltage dependent, and K^+-sensitive. The authors established the activity ratio of EAAT1:2:3 as approximately 1:3:6 [152].

To date, two Na^+-dependent glutamate transporters from rat brain, GLAST1 [197] and GLT-1 [165], along with the rabbit brain transporter EAAC1 [103] have been isolated and cloned. Interestingly, homologues of each of these transporters have been identified in the human brain [15] as EAAT1 (GLAST), EAAT2 (GLT-1), and EAAT3 (EAAC1). In addition, EAAT4 in the human cerebellum [68] and EAAT5 [14] in the human retina have been isolated. Moreover, the facilitative transport of glutamate has been described across the luminal [26] membrane, and it probably permits blood-EC glutamate transport.

The above-mentioned data suggest some actions of glutamate that occur in the CNS. It is thought that extracellular glutamate is transported into astrocytes, neurons, and ECs by at least three transporters. While glutamate in astrocytes is converted into glutamine

and released, it can be stored in neurons for synaptic transmission. In ECs, combined glutamate influx and glutaminase-derived glutamate quickly increases its intracellular content to levels exceeding the plasma concentration. In such cases, facilitative transport at the luminal membrane expels glutamate into the blood [26]. Blood-EC transport is also possible, but further movement would be difficult because of the high Na^+ gradient between the ECF (extracellular fluid) and ECs [34]. Additional difficulty results from the absence of facilitative transporters at the abluminal membrane.

Gathering those facts, the BBB is actively involved in the maintenance of CNS glutamate homeostasis. In hypoxia or ischemia, neurons and astrocytes depolarize and glutamate transporters running in reverse to increase of extracellular glutamate concentration [204]. Along with metabolic slowing, acidosis further upregulates astrocytic reverse transport [75]. The ECF glutamate concentration may reach almost toxic levels. At this point, EC transporters have the opportunity to remove excess glutamate and maintain its non-toxic levels; they therefore act as a protective mechanism against glutamate neurotoxicity [152].

The barrier and brain-blood neurotransmitter transport

γ-Amino-isobutyric acid (GABA) transport

The BBB used to be considered as a retention pool for neurotransmitters. Recently, GABA efflux through the BBB from the brain has been shown [205], suggesting that the BBB also acts in the regulation of neurotransmitters. Experiments with TM-BBB and the mouse brain capillary enriched fraction showed the expression of GAT2/BGT-1 (betaine/GABA transporter-1 corresponding to GAT2 in mouse) but no neuronal or glial transporters (GAT1 and GAT3). Those results suggest that GABA transporters expressed at the BBB differ from those expressed in neurons and glia. Further experiments showed the endothelial localization of GAT2/BGT-1 [205] and suggested its role as a BBB efflux transporter of GABA.

Norepinephrine and serotonin transport

Other transporters present in mouse brain capillary endothelial cells (BCECs) include the norepinephrine transporter (NET, localized at the abluminal membrane) and serotonin transporter (SERT, localized to both the luminal and abluminal membranes) [225]. Those transporters might work as a neurotransmitter-inactivating system in the vicinity of brain capillaries. This supposition was made on the basis of the hypothesis that brain microvasculature is regulated by both monoamines released from adrenergic and serotonergic neurons as well as the expression of adrenergic and serotonergic receptors in brain microvessels [44, 59, 112, 175].

The function of the luminally-localized SERT remains to be elucidated. Based on pharmacological serotonin function, one may emphasize SERT's role in maintaining cerebral blood flow by serotonin clearance from cerebral intravascular space to inhibit serotonin-enhanced blood coagulation. Similarly, Ganapathy et al. [71] suggested that SERT localized at the human brush-border membrane facing the maternal blood may be involved in maintaining uteroplacental blood flow [20, 71].

The barrier's function in the maintenance of CNS conditions

It seems logical that factors influencing the CNS may regulate the BBB and its proper barrier functions. Taurine (2-aminoethanesulfonic acid) is thought to play a role in CNS development, neuromodulation, and osmoregulation. Because of its limited biosynthesis, dietary taurine is important in maintaining its proper level. Studies on primary cultures of bovine BCECs revealed that a taurine transport system exists on both the luminal and abluminal sides of BCECs [210], supporting the hypothesis of its involvement in brain taurine supplementation. Furthermore, hypertonic conditions induce taurine uptake and taurine transporter (TAUT) mRNA transcription in TR-BBB cells [104]. This may mean that TAUT, in addition to ATA2 (System A isoform), is involved in CNS osmoregulation.

The increase of ISF taurine levels during ischemia has been described to exert a neuroprotective effect [69, 127]. Studies revealed that TNF-α induces the uptake of taurine and its transporter mRNA transcription in TR-BBB cells, and TNF-α is induced in brain ischemia or traumatic injury. Therefore, the up-re-

gulation of BBB taurine transport may occur in brain cell damage.

The barrier and energy metabolism

The brain cannot store its main energy substrate – glucose. BBB cells express glucose transporter 1 (GLUT1), which therefore plays a crucial role in glucose supplementation to the brain [48]. For energy homeostasis, however, energy storage is necessary in addition to synthesis.

Creatine seems to play such role in the brain, and its concentration in the brain is about 180-fold higher than that in the plasma. Energy is stored in phosphocreatine as ATP-derived phosphate sufficient for ATP's regeneration from ADP when needed [126]. Creatine biosynthesis takes place mainly in the kidney and liver, and it can be supported by dietary supplementation. Therefore, the circulating blood pool supplies muscle creatine *via* the creatine transporter (CRT). Conversely, this route seems to be limited for the brain [53]. Ohtsuki et al. [147] showed that conditionally immortalized mouse brain capillary endothelial cells (TM-BBB) have a creatine transport system mediated by CRT. This system allows the BBB to accumulate creatine against its concentration gradient [89, 147, 212]. Those results suggest two roles for the BBB in the energy homeostasis of the CNS. It acts as both a pathway for the energy source as well as a pathway for the energy buffer. Defects in either system can cause alterations in energy homeostasis followed by CNS dysfunction.

BBB studies on physiological functions revealed new regulation and transport systems. Regarding those facts, it seems clear how important the BBB is for maintaining the proper CNS environment; its significance becomes even greater during pathological conditions [9]. Further studies are needed, however, for complete elucidation of its complex role [149].

Cellular interactions at the BBB

Describing the BBB's structure as a cell complex implies more than a morphological set of few types of cells and their surrounding structures. The cells forming BBB depend on themselves not just anatomically and spatially but also biochemically and autocrinically. The BBB is a biochemical-anatomical net that permits wide cellular cross-talk [21]. Due to the close contact of ECs and astrocytes, the local concentration of secreted substances is high. Three-dimensional microanatomical visualizations of glial-endothelial interactions suggest the existence of specific "microdomains," in which one EC is bound to one astrocyte [101]. Thus, it becomes apparent that microvessels, neurons, and glia are organized into perfectly planned neurovascular units regulating cerebral blood flow [91]. In these units, even the proper spatial (apical/basal) polarity of EC/astrocyte composition is significant [1].

Many experiments confirm the important role of astrocytes in BBB formation and functioning at the physical, transportation, and metabolic levels [38, 54, 84, 97, 128, 169, 177, 196]. Experiments revealed that astrocytes are key in the formation of tube-like structures from ECs and pericytes. These experiments suggest that direct EC-astrocyte connections are indispensable for the morphogenesis and development of a vessel wall [169]. The involvement of other cells present at the BBB has also been shown in barrier development [27, 61, 62, 88, 169, 183, 235], and so has the influence of ECs on astrocytes [67, 133]. Furthermore, the up-regulation of the expression of many transporter systems, including γ-glutamyl transpeptidase (γ-GTP), GLUT-1, L-system, A-system, and Pgp, have been observed in ECs to exhibit glial influence [24]. Reversed influence has also been observed.

The list of astrocyte-originating factors essential for the proper differentiation of brain endothelial cells is still growing [2]. Evidence indicates that astrocytes-derived factors like TGF-β are responsible for the decrease in expression of the endothelial tissue plaminogen activator (tPA) and thrombomedullin (TM) genes. In addition, GDNF (Glial Derived Neurotrophic Factor) is responsible for BBB maturation. Moreover, angiopoetin 1 (ANG1) acts on the TIE2 endothelium-specific receptor tyrosine kinase 2. A role has also been assigned to basic fibroblast growth factor (bFGF), interleukin-6, and hydrocortisone [1, 74, 92, 118].

Another remaining question centers on how the brain endothelium influences astrocytes and neuron precursor cells. ECs are the source of a variety of factors that induce astrocyte differentiation. *In vitro* ex-

periments with EC/astrocyte co-cultures show this chain of dependence. For example, it has been shown that leukemia inhibitory factor (LIF) deriving from astrocytes causes the differentiation of astrocytes [133, 233]. Furthermore, two-way reliance between ECs and astrocytes causes the up-regulation of aquaporin-4 in astrocytic end feet [171], and the induction of antioxidant enzymes is mediated in both types of cells [189]. Furthermore, γ-glutamyl transpeptidase, an endothelial enzyme, is also activated in cooperation with astrocytes [137].

Dividing neurons also depend on ECs. It has been observed that dividing localize in richly vascularized regions. More than one-third (37%) of these cells are immunoreactive for endothelial factors [158].

Recent experiments with immortalized EC line hCMCE/D3 cells supported the hypothesis of astrocytic involvement in functional BBB development. It has been shown that treatment with cAMP-inducing agents and coculture with primary human astrocytes upregulates the expression of claudin-5 and occludin by ECs, thereby contributing to the development of the functional BBB [228].

Evidence sugests that ECs and astrocytes express a variety of receptors for numerous agents able to modulate brain functioning [79, 160]. Moreover, many of those agents derive from astrocytes and ECs themselves (e.g., ATP, endothelin-1, glutamate, IL-1β, IL-6, TNF-α, MIP-2, NO, and I2). The signaling network working within the neurovascular unit is thus multifaceted and often rapid in action [12, 78]. Physiological signaling that modulates BBB permeability may also become an advantage. For example, tightening of the barrier could be crucial in stress or hypoxia (e.g., because increased cAMP level causes a rise in resistance and upregulates Pgp), whereas its loosening may allow the passage of growth factors and antibodies (e.g., because nerve cells derived histamine mediates transient barrier opening) [1, 111]. Junctional protein expression can be altered by the transcription factor NF-κB and thus it can influence BBB permeability [39]. Furthermore, numerous mechanisms (e.g., ATP, histamine) influencing brain endothelial amino acids and glucose transport. Evidence indicates that factors released by astrocytes exposed to hypoglycemic conditions are able to enhance glucose uptake by brain ECs [172]. Cellular signalling pathway involved in the modulation of such BBB properties may depend on alterations in intracellular Ca^{2+} levels [4, 139, 157, 161] mediated *via* serotonin, glutamate receptor activation, or mechanical stimulation [29, 47, 157]. In addition, the possibility of ATP-mediated cross-talk involving ECs and intercellular gap junction suggests an astrocytic or neuronal signal propagation pathway [157]. This pathway can result in various nutritional or metabolic responses of ECs [35, 121, 129].

BBB and mineral/water balance of the CNS

In addition to all above-mentioned interactions and functions, astrocytes and their end feet play a role in the maintenance of ionic and water brain homeostasis [193]. Due to the high number of K^+ channels (Kir4.1, *rSlo*KCa) [167], they are appropriate for spatial buffering and deposition of K^+ ions in the perivascular space. From there, ions can be recycled when needed. K^+ uptake processes result in water influx and subsequent cell swelling. Astrocytes assist in the redistribution of this water *via* AQP4 water channels localized at their end feet [6]. Another contribution of astrocytes to water homeostasis involves the water influx accompanying glutamate uptake [193].

Multiple studies revealed that brain pericytes influence numerous EC functions [13, 52, 57, 85, 88, 181]. For example, they contribute to increased TEER and lowered barrier permeability [57]. Recently, it has been shown that a soluble factor released from pericytes (most likely angiopoietin-1) is responsible for the upregulation of occludin expression *via* tyrosine phosphorylation of the Tie-2 receptor [88].

Furthermore, studies revealed that pericytes cocultured with brain ECs downregulate expression of tissue plasminogen activator (tPA) *via* action of a soluble factor. They also upregulate the release of plasminogen activator inhibitor-1 (PAI-1), a known inflammatory stimulus. They abundantly express antithrombin and antifibrinolytic molecule PN-1. These facts suggest that pericytes regulate endothelial fibrinolysis [110]. In addition, pericytes seem to be involved in endothelial differentiation, as evidenced by astrocyte/EC/pericyte coculture studies in which they induced the termination of differentiation and vessel maturation. Finally, they may also be implicated in anti-apoptotic mechanisms that support the survival of capillary-like structures during culture conditions [169].

Junction tightness regulation

ECs also regulate TJ tightness and paracellular transport. Signal transduction takes place at the level of TJs, and two pathways may be distinguished. Signals are transferred from inside the cell to TJs, as well as from TJs into cell to influence gene expression, cell proliferation, and differentiation [131].

The transcellular signaling pathway regulating TJ tightness has not yet been completely elucidated. Numerous substances and signaling tracks, such as Ca^{2+}, protein kinase C, G protein, calmodulin, cAMP, phospholipase C, and tyrosine kinases, are believed to play a role in this system [19, 163]. Apart from the possibility of these signals being involved in TJ assembly and disassembly, they may also contribute to the regulation of cytoskeletal rearrangements [135].

It has been shown that ZO-1 is a substrate of a serine kinase [18] and PKC, which is crucial for the formation and regulation of TJs [198]. ZOs may thus be a scaffold for PKC signal transduction on the cytoplasmic side of the junction [230]. Furthermore PKCζ and PKCλ, atypical isotypes of PKC, are expressed within TJs. Along with ASIP (atypical PKC isotype specific interacting protein), it is thought that both play a role in the establishment of cellular polarity crucial to BBB functioning [96]. TNF-α-induced transcription of intercellular adhesion molecule 1 (ICAM-1) at the BBB mediated by NF-κB is also regulated by PKCζ [168].

Studies revealed that G-proteins influence the assembly of TJs and thus take part in TJ formation and regulation [18]. The $G\alpha_{12}$ subunit colocalizes with PKCζ at ZO-1 intercellular contacts [60] and is implicated in the development of electrical resistance across the membrane [56]. Furthermore, increased paracellular diffusion is correlated with rearrangements in perijunctional actin [176], and studies have revealed that small GTP-binding proteins are involved in this process.

Increased cAMP levels result in increased tightness of the junction [179]. Still, the outcome of cAMP reduction is a decrease of resistance. Therefore, the existence of proteins phosphorylated by protein kinase C, whose phosphorylation state regulates the cell to cell potency of adhesion, was proposed [114]. Furthermore, studies revealed that the reconstruction of TJs after disruption is connected with the upregulated phosphorylation of occludin [217]. Interestingly, the inhibition of tyrosine phosphatase in already existing TJs causes increased permeability associated with occludin proteolysis [227].

TJ-associated proteins are not the only players in signaling pathways regulating junctional tightness. Experiments have suggested that β-catenin is involved in the expression of Wnt target genes through interactions with the Lef/tcf family of transcription factors [164]. Furthermore, growth factors stimulate the dissociation of the β-catenin-VE-cadherin-PECAM-1 complex from the cell membrane. This allows further stimulation of gene expression *via* disengaging β-catenin.

Conclusions

Our knowledge about the structure and function of the BBB and its particular components is still growing. The importance of studies examining the basic aspects of the BBB is difficult to overestimate. However, much work has to be done to fully understand the BBB and permit the practical implementation of this information into clinical usage. In numerous circumstances, the permeability of the BBB increases as a result of drug action. The increase of BBB permeability during some disorders (e.g., neoplasms) is also important, because it may be possible to diagnose diseases much earlier, define tumor sizes, and properly plan for surgical procedures.

Another practical problem is the delivery of drugs to the CNS. Efforts are underway to develop strategies increasing the BBB permeability of important and potentially very effective new therapeutic approaches. Pathophysiological and therapeutic aspects of the BBB should be the subject of another review.

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Received:
March 5, 2008; in revised form: September 9, 2008.