Established in 1871
Swiss Medical Weekly
Formerly: Schweizerische Medizinische Wochenschrift
The European Journal of Medical Sciences

Review article: Medical intelligence | Published 12 November 2013, doi:10.4414/smw.2013.13892
Cite this as: Swiss Med Wkly. 2013;143:w13892

Breaking and building the wall: the biology of the blood-brain barrier in health and disease

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Summary

The blood-brain barrier (BBB) is a complex feature of brain endothelial cells that restricts the passage of blood-borne molecules into the brain parenchyma, while ensuring the delivery of essential nutrients and selected biomolecules. Brain vasculature is anatomically distinct from that of other organs and comprises in addition to endothelial cells, pericytes and astrocytes, which collectively form the neurovascular unit (NVU). This review focuses on the regulation of BBB properties by the NVU and the periphery. A brief overview of cellular components of the NVU, and BBB characteristics will be provided, with more emphasis placed on the molecular mechanisms involved in the development of brain vasculature and human genetic diseases primarily affected by dysfunction of components of the NVU. In addition, the regulation of brain vasculature by peripheral factors such as diet and systemic disease is discussed.

Key words*: blood-brain barrier; neurovascular unit; endothelium; pericytes; astrocytes; brain pathology*

Introduction

The human brain comprises only 2% of the total body weight, but consumes 20% of the body's metabolic energy. In response to synaptic activity, blood flow within the brain increases to meet the increased need for oxygen and nutrients. Thus, proper functioning of the brain vasculature is critical for the maintenance of optimal brain function. The brain parenchyma is separated from blood within the cerebral vasculature by the blood-brain barrier (BBB), and from cerebrospinal fluid (CSF) by the CSF-brain barrier and arachnoid-brain barrier. The BBB is a collective term for brain-specific endothelial cell characteristics that restrict the passage of blood-borne molecules into the brain, but ensure delivery of essential nutrients and selected biomolecules. In the animal kingdom, the BBB is already present in invertebrates and it is hypothesised that the BBB has developed to maintain ionic homeostasis around synapses [1]. Also, most neurotransmitters show low BBB permeability, which allows separation of the peripheral from the central pool of neurotransmitters. In invertebrates the BBB is glial based. For example, in the fruit fly (*Drosophila*), which has an avascular brain, perineural glia separate haemolymph and CNS. In phylogenetically more advanced invertebrates such as Crustacea and Cephalopods, the CNS is already vascularised and the BBB is formed by perivascular glial cells [2]. During evolution, the barrier function shifted to the endothelium, which is specialised to control the exchange between the blood and brain, whereas glial cells are specialised to control the local ionic environment [2].

The principal features of the vertebrate BBB are closed cell-cell junctions, a low rate of transcytosis and the expression of various solute carriers (SLCs) and adenosine triphosphate-binding cassette (ABC) transporters. In addition to these special molecular characteristics, the blood vessels in the brain are anatomically distinct. The first difference relates to the number and coverage of vascular mural cells. The capillary bed of the brain vasculature has a high density of pericyte coverage with full longitudinal coverage by pericyte processes. In contrast, the vascular smooth muscle cell coverage of arterioles and venules in the brain is similar to other organs. The second difference is that brain vasculature is covered by glial processes, the so-called astrocyte end-feet. Another distinct characteristic is the presence of Virchow-Robin spaces around penetrating arterioles that reside between the endothelial and glial basement membrane. In addition, brain vessels are contacted by microglia and nerve endings. The term neurovascular unit (NVU) is often used to describe brain blood vessels to underline the intimate physical and functional connection between the brain tissue and blood vessels.

Another special feature of brain vessels is that they are immunologically quiescent. Specifically, the expression of leucocyte-adhesion molecules is low and few peripheral leucocytes enter into the brain parenchyma.

In this review I will give a brief overview of the cellular components of the NVU, BBB characteristics, and discuss in more detail new insights about the molecular mechanisms involved in the development of brain vasculature. In addition, I will provide examples of human genetic diseases where dysfunction of components of the NVU is the primary cause of the observed brain pathology. In addition,

the regulation of brain vasculature by peripheral factors such as diet or systemic disease is discussed.

Cellular and acellular components of the neurovascular unit: development and homeostasis

Endothelial cells

Endothelial cells that line the luminal side of blood vessels enter the brain parenchyma early during neural tube development. In the mouse, this takes place at embryonic day 9.5, starting in the hindbrain region [3]. The CNS is vascularised by angiogenesis: by invasion of endothelial cells from the pre-existing perineural vascular plexus. The molecular mechanisms that drive the vascularisation of neural tissue are not fully understood. The master regulator of vasculogenesis and angiogenesis, vascular endothelial growth factor-A (VEGF-A), is upregulated by hypoxia and secreted by neural cells, and serves as a chemoattractant for vascular endothelial cells [3].

There is little known about the organ-specific signals required for the vascularisation and differentiation of the endothelium. However, recent years have witnessed considerable progress in our understanding of molecular mechanisms regulating brain vascularisation. Additional molecular players that act in concert with VEGF-A, and are required for brain angiogenesis have already been identified. For example, expression of the tumour necrosis factor family receptors TROY and death receptor 6, essential for VEGF-induced vascular sprouting, is induced by the Wnt signalling pathway [4]. Whereas VEGF-A signalling is important for the formation of vasculature in all organs, several brain-specific signalling pathways responsible for brain vascularisation have been identified. During early development, brain-specific vascularisation signals are derived from developing neural tissue. For example, the canonical Wnt signalling induced in endothelium was specifically shown to be important for brain vascularisation. Deletion of ligands Wnt7a/7b from neuroepithelial cells results in defective brain angiogenesis and improper differentiation of brain endothelium [5, 6]. Several other molecules either expressed or secreted by neuroepithelium regulate BBB development and integrity (e.g. presenilin-1, retinoic acid) [7, 8].

Another important signalling pathway for brain vascularisation is the transforming growth factor-β (TGF-β) pathway. Targeted deletion of the integrin β8 chain from neuroepithelium results in defective brain vascularisation [9]. It has been suggested that integrin αVβ8 expressed by neuroepithelium is needed for TGF-β activation [10]. The TGF-β signalling in endothelium and pericytes is crucial for angiogenesis and vessel maintenance (reviewed in [11]). Consistent with this, it was recently demonstrated that expression of *Tgfrb2* or *Alk5* on endothelium and not on neuroepithelium was needed for proper CNS vascularisation [12]. There are also endothelial-specific proteins that regulate brain angiogenesis in a cell-autonomous manner. The endothelial expression of GPR124 was shown to be required for brain vascularisation by regulating endothelial cell sprouting, migration and organ-specific differentiation [13–15]. Interestingly, the expression of GPR124 on endothelial cells is upregulated by TGF-β signalling [14].

The developing brain vasculature displays most of the BBB characteristics very early in embryogenesis and is largely impermeable to plasma proteins [16]. There are indications that brain vasculature may possess regional differences or at least different molecular mechanisms that guide brain vessel development during embryonic development [13–15].

As discussed above, the BBB characteristics of brain endothelium are induced by neural tissue of the developing brain. Additionally, maintenance of BBB characteristics is achieved by signals emanating from other cell types surrounding the endothelium, as will be exemplified below, and this could explain why brain pathologies are associated with breakdown of the BBB [17]. Little is known about the turnover of cells at neurovascular unit; however, all endothelial cells have the potential to proliferate in response to angiogenic signals. The turnover of endothelium in normal tissue is measured in years [18].

Vascular smooth muscle cells and pericytes

Vascular smooth muscle cells (VSMCs) and pericytes, collectively known as mural cells, enter the brain parenchyma together with endothelial cells during angiogenesis. It is of note that mural cells in the CNS have a distinct developmental origin depending on anatomical location. In the anterior part of the brain, the mural cell population is derived from the neural crest and in the posterior region, from the mesenchyme [11]. It is not known if this leads to functional differences between these two developmentally different mural cell populations.

Vascular smooth muscle cells surround small arteries, arterioles and venules. It is believed that the blood flow of brain vasculature is controlled at the level of arterioles, and thus involves vessel constriction and relaxation controlled by neurones and astrocytes that are mediated by VSMCs [19]. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) is autosomal, dominant, and represents the most common form of small vessel disease caused by the mutations in the *NOTCH3* gene [20]. Patients suffer from transient ischaemic attacks and strokes at a very young age and subsequently develop dementia. The clinical manifestations are restricted to the brain, but arteriopathy can be seen in other organs as well. In the normal brain, Notch3 is expressed by all mural cells, VSMCs and pericytes [21]. The pathogenesis of CADASIL is not fully understood, but aberrant Notch3 protein causes vascular fibrosis and VSMC degeneration, which leads to ischaemic stroke [19].

Pericytes are perivascular cells that surround the endothelium around capillaries [11]. Pericytes share the basement membrane and are in direct contact with endothelium. The CNS vasculature has almost 100% longitudinal pericyte coverage. During development, the platelet-derived growth factor-B (PDGF-B) / PDGF receptor β (PDGFRβ) signalling axis is important for pericyte recruitment and investment along developing vessels [11]. Maturation of endothelial cells and pericytes during angiogenesis are intimately linked, and defects in one cell type cause defects in the other [22]. For example, abnormal endothelial polar-

isation during development leads to poor recruitment of pericytes [23]. Older BBB reviews propagated the view that pericytes regulate the BBB at the level of endothelial junctions, an assumption based on a few *in-vitro* studies. However, careful analysis of cerebral vasculature and the BBB characteristics of pericyte-deficient mice have shown that pericytes regulate the BBB at the level of endothelial transcytosis during early development and in adults [24, 25]. Absence of pericytes opens an endothelial transcytosis route that lacks selectivity to the size and chemical nature of tracers [24]. Furthermore, pericytes regulate the expression of endothelial adhesion molecules on the developing vasculature, thus potentially regulating immune cell trafficking [25].

Striking pathological changes observed in the brains of pericyte-deficient animals lead to the formation of region-specific calcium deposits [26]. Interestingly, the extent of pericyte deficiency and BBB permeability correlates with the severity of calcified lesions observed in the brains of adult pericyte-deficient animals [26]. Thus, disturbances at the BBB lead to changes in brain homeostasis. PDGFRβ loss-of-function mutations were recently shown to represent one of the causes of familiar idiopathic basal ganglia calcification (IBGC) [27]. IBGC is a rare autosomal disease with dominant inheritance, which manifests with bilateral calcifications in various brain regions, mostly in the basal ganglia. Clinical features include migraine, Parkinsonism, seizures, cognitive impairment and ataxia [28]. Some mutation carriers, despite the presence of calcified lesions in the brain, remain asymptomatic [28]. The underlining cause of brain pathology of IBCG is not understood. The calcified lesions are associated with blood vessels and not within the neuropil [29]. It would be interesting to examine if affected individuals exhibit pericyte deficiency and altered BBB characteristics. It is intriguing that several other genetic diseases affecting brain vasculature (discussed below, e.g. band-like calcification with simplified gyration and polymicrogyria, Alexander disease) or causing neuroinflammatory disorders (e.g. Aicardi–Goutières syndrome) or neurodegeneration (e.g., Parkinson's disease) are associated with intracranial calcifications [30].

Astrocytes

Astrocytes are CNS macroglial cells that provide another distinct characteristic of brain blood vessels – certain astrocytes ensheath the entire abluminal side of blood vessels with specialised processes called astrocyte end-feet. Astrocyte end-feet are slender processes, enriched with water and potassium channels (e.g. aquaporin4 [Aqp4] and Kir 4.1, respectively) that are anchored to the basement membrane of blood vessels via the dystroglycan complex [31].

Astrocytes are specified in the CNS relatively late during development: in mice this takes place around birth, after neuronal differentiation and initial CNS angiogenesis has taken place [32]. Investment of blood vessels with astrocyte end-feet takes place postnatally and is completed two weeks after birth in mice [33]. Early studies have suggested that astrocytes are important for inducing the BBB properties of brain endothelial cells [34]. However, astrocytes are unlikely to be important in the early induction of BBB properties of brain endothelium, since developmentally, they differentiate later. Nevertheless, astrocytes are important regulators of BBB properties. As yet, relatively little is known about endothelium-astrocyte crosstalk and, even today, only few studies have addressed which astrocyte-derived signals regulate BBB development and maintenance. In addition, the cellular and molecular mechanisms leading to astrocyte end-feet formation and polarisation largely remain elusive. However, a few clues are merging: bone morphogenetic protein receptor type 1 (BMPR1A) signalling in astrocytes is necessary for the investment of astrocyte end-feet around vasculature [35], and pericyte-derived signals regulate astrocyte end-feet polarization [24]. Astrocytes have also merged as regulators of brain endothelial immune quiescence. Astrocyte-derived Sonic hedgehog (Shh) is needed for the active maintenance of endothelial immune quiescence and the Shh-activated pathway in endothelium has been shown to be deregulated in multiple sclerosis patients [36]. The targeted elimination of astrocytes that form astrocyte end-feet in experimental animals is currently not possible. Astrocytes are a heterogenous population and understanding of the specific function of different astrocyte populations first requires identification of the population-specific promoters to better understand astrocyte functions also at the NVU. Some clues regarding the role of astrocytes *in vivo* in regulating the BBB come from two human genetic diseases that specifically affect astrocytes and cause BBB dysfunction.

One of the diseases that primarily affect astrocytes is Alexander disease, a fatal neurodegenerative disease with an autosomal dominant inheritance pattern. It is caused by mutations in the *GFAP* gene leading to intracellular accumulation of glial fibrillary acidic protein, GFAP (Rosenthal fibres), which results in astrocyte death [37]. Alexander disease onset (neonatal, juvenile, late) and severity of clinical manifestations varies [37]. Neurodegeneration in Alexander disease is accompanied by BBB disruption and capillaries show an increase in pinocytotic vesicles and occasional damage of the endothelial basement membrane [38].

The second disease that potentially affects astrocytes and results in BBB impairment is megalencephalic leucoencephalopathy with subcortical cysts (MLC), which is a rare leucodystrophy caused by mutations in *MLC1* or *GLIALCAM,* and is characterised by chronic white matter oedema [39, 40]. MLC1 is predominantly localised to astrocyte-astrocyte contacts at astrocyte end-feet. The function of this gene is not known; however, it is predicted that *MLC1* encodes a transmembrane protein that could play a role in channel or transport function, and regulate water transport in astrocytes [40].

In the periphery, excess of interstitial fluid is removed from tissue by the lymphatic system [41]. The brain lacks lymphatic vessels and thus lacks a specific pathway for interstitial fluid clearance. However, recent research has identified a so-called "glymphatic" pathway (derived from glia and lymphatic) that facilitates movement of CSF from the subarachnoid space along paravascular spaces that surround penetrating arterioles. The clearance of interstitial fluid occurs along venous drainage pathways [42]. The authors of this study showed that the expression of water channel Aqp4 on astrocytes facilitates interstitial solute

clearance, suggesting that the bulk of interstitial fluid flow is supported by astrocytic water transport [42]. Interestingly, this pathway has been shown to transport amyloid β from the brain into blood. The existence of interstitial fluid drainage in the brain has been suggested before; however, the drainage pathway was thought to be located only along capillaries and arteries, and the flow of interstitial fluid was considered to be opposite to the direction of blood flow [43].

Thus, emerging data suggest that astrocytes at the NVU are important regulators of brain fluid transport and that disturbances in astrocyte end-feet function are associated with oedema.

Perivascular macrophages

Perivascular macrophages are blood-derived phagocytotic cells that reside in the perivascular spaces which surround penetrating arterioles and are continuous with the subarachnoid space (so-called Virchow-Robin space) [44]. These perivascular cells perform phagocytic functions and regulate the immune response and lymphocyte entry into brain parenchyma [45, 46].

Microglia

Microglial cells, as the name suggests, are of small size, but the term is actually a misnomer, since they are now considered to be the resident macrophages of the brain and spinal cord.

Microglial cells invest the brain very early during development, just before the endothelial cells invade and vascularise the tissue. Developmentally, microglia are derived from the yolk sac and thus have a different developmental origin from perivascular macrophages residing in the Virchow-Robin space [47, 48].

The biological functions of microglia during development and the maintenance of the BBB and NVU has not been vigorously investigated. In the adult organism, microglia are important for maintaining endothelial integrity by supporting damaged vessels and facilitating endothelial repair in homeostasis and pathological conditions [48, 49]. The molecular signalling pathways by which microglia support the integrity of brain vessels remain elusive.

Basement membrane

Basement membrane is an important regulator of all epithelial cells, and is essential for maintaining tissue integrity. It is also crucial for the integrity of brain vasculature. The main basement membrane components are fibronectin, collagen IV, laminins, perlecans and nidogens [50]. The basement membrane can be divided into two distinct parts that have a different composition and cellular origin. The endothelial basement membrane is deposited by endothelial cells and pericytes/VSMCs, and the parenchymal basement membrane by astrocytes [51]. Although the role of the individual matrix molecules in the biology of blood vessels is far from understood, some recent studies offer insights. Mouse genetic studies have shown that basement membrane is important for maintaining vessel stability and the lack of individual matrix components is a cause of haemorrhages due to vascular fragility [50]. In humans, genetic defects in the gene encoding a component of collagen IV (*COL4a1, COL4a2*) lead to nephropathy and haemorrhagic stroke [52–54]. Besides providing mechanical stability, the basement membrane is also essential for endothelial cell polarisation and lumen formation [23]. Proper endothelial polarisation is needed for the correct localisation of influx and efflux transporters. The vascular basement membrane components agrin and laminins are important for anchoring astrocyte end-feet proteins Aqp4 and Kir 4.1 via dystroglycan complex [31]. Merosin-deficient congenital muscular dystrophy is an autosomal recessive disorder caused by mutations in the *LAMA2* gene that encodes a laminin alpha 2 chain [55]. Magnetic resonance imaging (MRI) analysis of the brain in patients with merosin-deficient congenital muscular dystrophy has shown the presence of vasogenic oedema [55, 56]. It should be interesting to determine whether in these patients the organisation of astrocyte end-feet is affected.

The blood-brain barrier characteristics of brain endothelium

Cell-cell junctions

Endothelial cell-cell junctions of the brain are the most intensively studied component of the BBB. In their seminal work published in 1967, Reese and Karnovsky demonstrated that the mammalian BBB is endothelium-based [57]. Endothelial cell-cell junctions are composed of two different types of junctions, adherens and tight junctions, which are established by transmembrane proteins mediating homophilic extracellular interaction, and are connected to the actin cytoskeleton inside the cell [58]. Of note, the integrity of the cytoskeleton is also important for maintaining BBB characteristics [59]. Adherens-type junctions are composed of vascular endothelial (VE-) cadherin, which is crucial for the integrity of all blood vessels in the body [58]. In addition, endothelial cells express neural cadherin, which is important for association with pericytes [60]. Intracellulary, adherens junctions connect to the cytoskeletal protein complex consisting of many proteins (e.g. α-catenin, β-catenin, plakoglobin, p120, etc.). The molecular composition of this cytoplasmic complex is important for maintaining vascular integrity, as demonstrated by studies where individual elimination of these proteins (e.g. p120, afadin) in endothelial cells resulted in embryonic death due to defects in vascular development [58]. Tight junctions are composed of several transmembrane proteins and the three main components are claudins, occludins and junction adhesion molecules (JAMs). In the cytoplasm, these molecules are associated with a number of cytoplasmic proteins including zonula occludens proteins [58]. It should be underlined that cell-cell junctions are present in all endothelial cells in the body; however, the junctions are closed in the brain and restrict the passage of even ions. The junctions are also needed to maintain endothelial polarisation and thus the correct localisation of BBB transport proteins. There are more than 20 claudins described, but only a few are expressed by brain endothelium (e.g. claudin 1, 3, 5). Today, little is known about the dynamic regulation of endothelial junctions and how individual junctional components interact with each other. However, the presence of VE-

cadherin-based adherens junctions has been shown to be crucial for the induction of tight junction components (e.g. claudin 5) [61]. Tight junctions are composed of many different proteins and there seems to be extensive plasticity in maintaining the closed junctions. For example, genetic elimination of occludin, one of the components of tight junctions, in mice does not result in an overt BBB phenotype [62]. In humans, mutations in the occludin gene cause an autosomal recessive neurodevelopmental disorder – band-like calcification with simplified gyration and polymicrogyria [63]. Interestingly, both patients with this mutation and mouse knockouts share similar features, with the development of intracranial calcifications that are deposited in the vicinity of blood vessels [63]. In humans, homozygous mutations of *JAM3* cause intracerebral haemorrhages, subependymal calcification and cataracts [64].

Influx and efflux transporters

Because of the closed state of brain endothelial junctions, brain endothelium is equipped with a transport system that not only provides a selective route for nutrients, ions and bioactive macromolecules, but also ensures elimination of toxic molecules. These transporters are expressed in a polarised manner, which allows the effective exchange of molecules and ions between blood and the brain parenchyma [65].

SLCs mediate influx of a variety of polar molecules into brain parenchyma. The SLC protein family consists of approximately 300 genes and brain endothelial cells express many different transporters that allow the influx of glucose, amino acids, nucleosides, neurotransmitters (e.g. serotonin, dopamine) and so on. It is intuitive that disturbances in SLCs expression or function may have devastating effects on brain development and function. Indeed, mutations in *GLUT1* gene, which encodes a facilitative glucose transporter 1 (GLUT1) expressed by brain endothelial cells, cause GLUT1 deficiency syndrome [66]. This syndrome is caused by GLUT1 haplo-insufficiency and leads to mental retardation accompanied by variety of neurological symptoms (e.g. ataxia) [66]. GLUT1 is expressed by brain endothelial cells very early during brain angiogenesis and is considered a feature of the early BBB. Recent studies in zebrafish have demonstrated that, at least in lower vertebrates, GLUT1 expression is also required for the formation of tight cell-cell junctions between endothelial cells during brain angiogenesis [67]. In addition to disturbances in nutrient delivery, defective transport of bioactive molecules can lead to a diseased state. SLC16A2 (MCT8) is a thyroid hormone (T3) transporter and dysfunction of this transporter causes Allan-Herndon-Dudley syndrome [68]. Allan-Herndon-Dudley syndrome is inherited in an X-linked recessive manner and thus mostly affects males. More than 12 different mutations have been described in humans so far, all leading to defects in thyroid hormone transport into brain tissue [69]. Thyroid hormone by regulating, among others, neuronal differentiation, myelination and synapse formation, is critical for brain development during embryogenesis [68]. Affected individuals present with congenital hypotonia with severe psychomotor delay [69].

The brain endothelium expresses many different ATP-driven efflux pumps that are localised on the luminal side of the endothelium [70]. The human genome encodes at least 48 ABC-transporters [71], which restrict passage of lipophilic xenobiotics that enter the endothelium via lipid-mediated transport. The main BBB efflux transporters are P-glycoprotein (ABCB1), multidrug resistance-associated proteins (Mrp1, 2, 4, 5) and breast cancer-related protein (Bcrp). The most studied example is the P-glycoprotein, which is polyspecific and regulates the efflux of about half of all commonly prescribed drugs. The expression of ABC transporters is influenced by inflammation, and reduced expression of P-glycoprotein is observed in neurodegenerative diseases (e.g. Alzheimer's, Parkinson's) [70].

Transcytosis

Peptides and large molecular weight proteins enter the brain via transcytosis. Transcytosis can be specific (receptor-mediated) or non-specific (adsorptive-mediated). Proteins and protein complexes that enter the brain via receptor-mediated transcytosis include Fe-transferrin, lipoproteins, immunoglobulin G, insulin and leptin [65]. The scarceness of endocytotic vesicles in brain endothelium has been documented extensively by electron microscopic analysis; however, the regulation of the low transcytosis rate in brain endothelium remains to be clarified. Interestingly, an increased number of endothelial vesicles is the first change seen in brain endothelium in response to hypoxia and has been described as an early step in endothelial injury during many pathological conditions (e.g. traumatic brain injury, stroke). Recent research has identified pericytes as regulators of endothelial transcytosis in the CNS (see above "Vascular smooth muscle cells and pericytes") [24, 25].

Blood-borne gases such as oxygen and carbon dioxide diffuse passively into the brain parenchyma along their concentration gradients. Accordingly, oxygenation and carbon dioxide removal are both blood-flow dependent [65].

Vascular diseases affecting the central nervous system

The list of diseases in which a vascular malformation or malfunction leads to vessel dysfunction, rupture and subsequent brain damage is relatively long and includes cerebral arteriovenous malformations, Moya-Moya syndrome and familial forms of small vessel diseases of the brain causing leucoencephalopathy, cerebral amyloid angiopathies, cerebral cavernomas, intracranial aneurysms, etc. Mutations in the *NOTCH3, COL4a1* and*COL4a2* genes that are responsible for hereditary vascular leucoencephalopathies were discussed above. Most likely, the recent advances in next generation sequencing and imaging technologies will facilitate the identification of new genes causing cerebral vascular diseases. For example, a novel form of dominantly hereditary vascular leucoencephalopathy was described that was mapped to chromosome 20q13 [72]. The precise pathological mechanism of many CNS vascular diseases is often poorly understood or not known at all. However, the identification of the disease causing gene(s) for several of these diseases has led to the generation of animal models, which will help clarify the pathogenesis and may lead to the generation of treatment strategies.

Familial cerebral cavernous malformations (CCMs) represent a category of diseases where enormous progress in understanding the genetic causes and molecular mechanism has been made during recent years. CCMs are vascular lesions that develop in the venous-capillary bed and are composed of mulberry-like clusters of endothelial channels surrounded by a thick endothelial basement membrane [73, 74]. CCMs are relatively common (1:200–250) and can be either sporadic or inherited. Familial forms of CCMs are inherited in an autosomal dominant manner and are caused by loss-of-function mutations in one of three genes: *CCM1*, *CCM2*, and *CCM3* [74]. Patients suffer from recurrent cerebral bleedings which cause neurological symptoms in little more than half of the CCM mutation carriers [73]. Despite considerable progress in characterising the functions of CCM proteins in endothelial cells, the underlying cause of pathological alterations in CCM mutation carriers is still not clear. CCM proteins regulate many aspects of endothelial biology (e.g. polarisation, branching, maturation), which all could contribute to the lesion formation [73].

The blood-brain barrier and neurodegenerative and neurological diseases

Breakdown of the BBB and NVU has been associated with many neurological diseases that are beyond the scope of this article. The reader is referred to several in-depth reviews [17, 75]. Furthermore, the so called vascular hypothesis postulates that vascular dysfunction is the primary insult common to neurodegenerative diseases like Alzheimer's [76]. Currently, unequivocal demonstration of NVU dysfunction as the primary cause of common neurodegenerative diseases is lacking. Additionally, various aspects of endothelial and NVU biology are altered in different neurodegenerative diseases, which most likely excludes a uniform, generic mechanism. For example, in Alzheimer's disease, the faulty clearance of the amyloidogenic amyloid-beta forms via the brain vasculature seems to be an important component of the disease pathogenesis. Similarly, regional alterations in vascular permeability in multiple sclerosis allow the ingress of peripheral leucocytes, and thus the loss of immune quiescence in the brain and spinal cord [76, 77].

The blood-brain barrier and peripheral diseases

Whereas there is increased interest in the changes in the BBB occurring in the setting of neurodegenerative diseases (e.g. Alzheimer's, amyotrophic lateral sclerosis), even less is known about changes occurring in brain vasculature due to peripheral diseases. Accordingly, I have listed a few examples of peripheral diseases, where changes in the brain vasculature are recognized to result in neurological complications. A mechanistic understanding of the BBB alterations in these peripheral diseases is missing.

Acute liver failure

Acute liver failure with a sudden loss of hepatic function triggers brain oedema, which is the main cause of death [78]. Although the older literature suggests that cytotoxic oedema is the major cause of brain oedema, recent studies have demonstrated that vasogenic oedema is an important pathogenic mechanism [79]. Interestingly, BBB breakdown is accompanied by subtle changes in the brain endothelium at the ultrastructural level. There is increased vesicularity in the endothelium and altered architecture of the endothelial cell-cell junctions [80–82]. It is believed that systemic factors such as inflammatory cytokines and ammonia are responsible for the changes in the BBB (reviewed in [78]).

Posterior reversible encephalopathy syndrome

The posterior reversible encephalopathy syndrome (PRES) is a recently described neurological syndrome characterised by headaches, visual disturbances and seizures. Recognized causes of PRES include chemotherapy, renal failure, pre-eclampsia and the administration of immunosuppressive agents [83].

The pathophysiology of PRES is poorly understood, but involves vasogenic oedema that is usually bilaterally symmetric, and localised to subcortical white matter of the parietal and occipital lobes, but may also involve cerebellum and brain stem [83]. Which component of the BBB and neurovascular unit is affected is unknown. Interestingly, if quickly diagnosed and treated, the oedema is reversible (as suggested by the name of the syndrome).

Postoperative cognitive decline and anaesthesia

Patients undergoing surgery can develop postoperative cognitive decline (POCD). Risk factors are advanced age and comorbidities (e.g. metabolic disease, neurological disease), but the pathophysiology of POCD is not well understood. One emerging concept suggests that POCD is caused by neuroinflammation evoked by peripherally produced proinflammatory cytokines in response to surgery [84]. A recent study suggests that even surgical procedures that do not involve the nervous system directly can induce BBB dysfunction that leads to exstravasation of peripheral monocytes causing neuroinflammatory changes and subsequent memory deficits [85]. Studies in animals have shown that isofluorane anaesthesia can cause region-specific BBB opening [86], but short-duration treatment does not cause memory impairment [87].

The blood-brain barrier and hypoxic conditions

The BBB is very sensitive to hypoxic conditions, and alterations in the BBB in response to hypoxia occur almost immediately. The appearance of pinocytotic vesicles is observed immediately, whereas the late effect is leakage through the necrotic vessel wall [88]. Hypoxia accompanies disorders like stroke, carbon monoxide poisoning, and cardiac arrest. Interestingly, carbon monoxide poisoning causes selective damage to the globus pallidus and cerebral white matter, indicating that certain brain areas are more vulnerable [89]. Also, high altitude is known to cause vaso-

genic cerebral oedema [90]; however, the pathogenic mechanisms leading to oedema remain elusive. Of potential significance is the upregulation of VEGF-A that has been shown to be associated with low oxygen levels and occurs after transient ischaemia [91, 92].

The blood-brain barrier and metabolism

As discussed above, dysfunction of the BBB causes many pathological conditions and is modified by diseases that are not primarily caused by BBB dysfunction. Very little is known about how metabolism influences the BBB at the molecular level in the healthy organism. Although this is a largely unexplored area, some insights are already emerging. For example, the expression of ABC transporters is increased at the transcriptional level by the presence of xenobiotics and the activity of P-glycoprotein can be regulated by dietary components [70, 93]. The expression of SLCs also changes in response to metabolic state. Under normal circumstances, D-glucose is the main energy source for the brain, but monocarboxylates can also be used, especially during the neonatal period, fasting and high-fat diets [94]. The expression of monocarboxylate transporters in the brain is regulated by obesity, high-fat diet and hyperglycaemia [95–97]. Interestingly, in hibernating animals that have a lipid-based metabolism, the brain uses ketone bodies as an energy source, which is accompanied by upregulation of MCT1 at the BBB [98]. Alternatively, fasting has been shown to regulate another component of the brain barrier, the brain-cerebrospinal fluid barrier, and to alter the characteristics of the BBB at the level of endothelial junctions. In response to lowered blood glucose, microvessels that surround the hypothalamic median eminence and reach the hypothalamic arcuate nucleus become fenestrated, thereby facilitating the access of circulating signals to brain nuclei regulating metabolism [99].

Conclusions and perspectives

From an evolutionary standpoint, the BBB is already present in invertebrates, and developmentally the BBB arises during the early phases of brain vascularisation. The molecular development and regulation of the BBB is currently under intense investigation. Clearly, a collective effort from research groups working in different fields (vascular biologists, physiologists, neurobiologists, etc.) is needed to better understand the development and homeostasis of complex structures such as the BBB and NVU. The brain vasculature and vasculature in general is no longer viewed as a simple conduit that delivers oxygen and nutrients and passively restricts passage of blood-borne molecules into the brain. Of note, the vasculature influences brain development and homeostasis and *vice versa* – neural tissue regulates the development of brain vasculature and its homeostasis.

The BBB and the NVU are complex structures that cannot be adequately represented in *in-vitro* models. Recent advances in the comprehensive understanding of the molecular basis of BBB regulation and cell-cell signalling pathways at the NVU can largely be attributed to increased studies using animal models. Although brain vascular development and deregulation during pathological conditions is better understood at the molecular and cellular level, the majority of specific questions are unanswered. Which molecular signalling pathways regulate the components of the BBB *in vivo*? How are BBB characteristics deregulated during pathological conditions? How does diet and lifestyle affect the BBB? How can the BBB be safely modified in order to increase an effective drug concentration in the brain? Hopefully, the next years will yield many "eureka moments" for scientists investigating the BBB, which will translate into the laboratory and clinical environments.

Acknowledgment: I would like to thank my colleagues Elisabeth Rushing, Maarja Andaloussi Mäe, Christer Betsholtz and Michael Hugelshofer for inspiration and discussions.
Funding / potential competing interests: No financial support and no other potential conflict of interest relevant to this article was reported.

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