REVIEW

Endothelial Control of Cerebral Blood Flow

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Since constant perfusion of blood throughout the brain is critical for neuronal health, the regulation of cerebral blood flow is complex and highly controlled. This regulation is controlled, in part, by the cerebral endothelium. In this review, multiple modes of endothelium-derived blood flow regulation is discussed, including chemical control of vascular tone, heterotypic and homotypic cell-cell interactions, second messenger signaling, and cellular response to physical forces and inflammatory mediators. Because cerebral small vessel disease is often associated with endothelial dysfunction and a compromised blood-brain barrier, understanding the endothelial factors that regulate vessel function to maintain cerebral blood flow and prevent vascular permeability may provide insights into disease prevention and treatment. (*Am J Pathol* 2021, 191: 1906–1916; <https://doi.org/10.1016/j.ajpath.2021.02.023>)

Cerebral blood flow (CBF) is tightly regulated to ensure maintenance of brain health and function by coordinated interactions of the cerebral vasculature with the surrounding tissue. Dramatic changes or disruptions in CBF can compress the intracranial tissue or result in ischemia and tissue death. The high metabolic needs of the brain require a large volume of blood flow, comprising almost 20% of the total cardiac output.¹ The regulation of CBF ensures sufficient delivery of nutrients and oxygen and removal of metabolic waste. To underline the importance of CBF regulation, normal brain functions halt within seconds when cerebral blood flow stops, resulting in critically damaged neuronal function.² To ensure the required perfusion, a dense network of blood vessels fills the brain. Cerebral small vessels such as small arteries, arterioles, venules, and capillaries, are crucial for maintaining blood flow. Cerebral small vessel disease (CSVD) is often associated with altered CBF, which can lead to increased blood-brain barrier (BBB) permeability and altered transport properties of the cerebral microvasculature. Factors controlling CBF are maintained by endothelium, a monolayer of specialized cells that line the blood vessel lumen. The endothelium is involved in numerous control processes, including regulation of vascular tone, inflammatory response, thrombosis, adhesion, and vascular permeability.³ The importance of endothelium

is demonstrated by the fact that endothelial dysfunction plays a recurring role in the pathogenesis of CSVD. This review focuses on the intricate role of the endothelium in regulating blood flow and vascular permeability in the brain.

Chemical Control of CBF

The CBF is controlled by numerous vasoactive mediators produced by the endothelium, including nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF), eicosanoids, and endothelin-1.⁴ Cerebral vascular tone is maintained by a balance of these mediators.⁵ Vascular tone is dependent on the contractile activity of the smooth muscle cells associated with the small arteries and arterioles.⁶ NO, the most studied endothelium-derived vasodilator, is responsible for inhibiting platelet activation and thrombosis, limiting adhesion of inflammatory cells, and regulating the progression of disease states, such as atherosclerosis.⁴ Significant reductions in NO lead to

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This article is part of a review series on small blood vessel disease in the brain, addressing current knowledge, new mechanisms, biomarkers, and therapeutic approaches.

platelet aggregation, increased proliferation of the associated smooth muscle, higher blood pressure, and compromised vascular relaxation.⁷ The metabolic product CO₂ can also affect NO production via endothelial G $\alpha_{q/11}$ proteins to mediated site-specific dilation or constriction, thus modulating CBF.⁸

NO is produced by nitric oxide synthase, which comes in several isoforms. The isoform in the endothelium is the endothelial NO synthase (eNOS). One route for eNOS production of NO involves the calcium-dependent binding of calmodulin; therefore, factors that control intracellular calcium regulate eNOS activity and NO production. In addition, eNOS is regulated by calcium-independent mechanisms, and activated by the sphingolipid ceramide.⁹ NO and other endogenous mediators alter the intracellular [Ca²⁺] of the smooth muscle cells, mainly through second messenger and K⁺ channel activation pathways that consequently cause smooth muscle contraction and relaxation.⁵ Up-regulation or activation of eNOS is protective during brain ischemia,⁷ and mouse models of partial eNOS deficiency manifest with thrombotic cerebral infarctions and vascular occlusions similar to the pathophysiology of Alzheimer disease.¹⁰ Therefore, the levels of endothelium-derived factors can impede or accelerate the progression of cerebral vascular diseases and must be finely controlled for healthy brain function.

EDHF, as part of the CBF control pathway, hyperpolarizes the vascular smooth muscle to induce relaxation of the vessel wall. A precise EDHF-mediated dilation mechanism is still under debate, but it is believed that changes in endothelial [Ca²⁺] mediate the EDHF response. As best understood, the EDHF pathway begins with a rise in endothelial intracellular [Ca²⁺] and concludes with smooth muscle relaxation. Evidence points to EDHF being more important for endothelium-dependent vasodilation with decreasing vessel diameter.¹¹ The dilatory effect of EDHF may be mediated, not as a true chemical mediator, but by myoendothelial gap junction signaling.¹² The EDHF-mediated response following ischemic injury has been compared with the NO vasodilatory response. In certain scenarios, EDHF and NO appear to have a compensatory relationship as NO dampens but does not completely suppress the EDHF-mediated response.¹³ In pathologic conditions, NO dilation tends to be significantly attenuated¹⁴; however, EDHF is up-regulated, suggesting that it plays a compensatory role in disease.¹⁵ As an example of one such disease condition, EDHF is increased in ischemic injury when vascular tone is considerably affected.¹⁶ Mechanistically, EDHF-mediated dilations after ischemia/reperfusion appear to result from augmented endothelial Ca²⁺ signaling.¹⁷ The cumulative data point to an important role for EDHF in disease states to rescue vascular tone and maintain CBF.

Vascular tone is also regulated by eicosanoids, a family of bioactive signaling lipids derived from the essential fatty acid arachidonic acid.¹⁸ Three key enzymes in

arachidonic acid metabolism have been identified for their role in the brain: cyclooxygenase (COX), lipoxygenase, and epoxygenase. These enzymes produce the metabolites thromboxane, prostacyclins, prostaglandins, epoxyeicosatrienoic acids, and leukotrienes. Although not restricted to the endothelium, these metabolites regulate vascular tone under both physiological and pathologic conditions. The activity of many of these enzymes increases during ischemia.¹⁹ In addition to smooth muscle contraction and relaxation, these metabolites are mediators of leukocyte function. Leukotriene B₄, which is a dynamic metabolite derived from arachidonic acid, is specifically involved in neutrophil recruitment after aneurysm.²⁰ The differential concentrations and localization of COX, lipoxygenase, and epoxygenase can regulate CBF. Of the three enzymes, the COX family of isoforms 1 to 3 are best understood in the cerebral vasculature. COX-1 maintains vascular tone as well as plays a key role in cerebral microcirculation vasodilation. Of the vasodilator metabolites produced by COX, prostacyclin and prostaglandin E₂ are predominant in healthy physiological states, where prostacyclin synthesis by COX-1 exhibits vasoconstrictor activity.²¹ COX-2 is induced by inflammatory stimuli and to promote a sustained inflammatory state.²² Reactive oxygen species is released during the COX-2 catalysis of arachidonic acid to prostaglandin E₂²³ and contributes to the overexpression of COX-2 under pathologic conditions.²⁴ This interaction between reactive oxygen species and COX-2 initiates a self-propagating onslaught of oxidative stress and inflammation. Because of this, COX-2 inhibitors are considered anti-inflammatory and maintain CBF by preventing vessel occlusion.

Endothelin (ET) is a vasoconstrictor peptide secreted by the endothelium that can significantly reduce local CBF.²⁵ It has been identified in three structurally similar isoforms (ET-1, ET-2, and ET-3), with ET-1 being the most potent vasoconstrictor in the regulation of CBF. ET-1 is the predominant form produced in endothelial cells by the endothelin-converting enzyme 1 and 2.²⁶ Two G protein-coupled receptors, endothelin receptor type A, and endothelin receptor type B, mediate the activity of ET-1. ET-1 has dual vasoactive effects, mediating vasoconstriction via endothelin receptor type A receptor activation of the smooth muscle compartment, and vasorelaxation via endothelin receptor type B receptor activation of endothelial cells.²⁷ On a transcriptional level, endothelin receptor type B receptors are up-regulated in the brain following ischemia, which further exacerbates the ischemic region; therefore, antagonists to endothelin receptor type B may be a therapeutic target.^{28,29} Endothelins are often associated with neurodegenerative disease. They are characterized by long-lasting vessel constrictions, which make them poorly suited for a dynamic regulation of blood flow. Therefore, endothelins likely do not control resting CBF but rather play a more important role in pathologic states when large changes in

CBF are required.^{28,30} In fact, several *in vivo* models of ischemia utilize the stereotactic injection of ET-1 to the brain to induce vasoconstriction and subsequent ischemia.^{31,32} As evidenced by the overexpression of ET-1 in inciting focal ischemia, the levels of endothelial-produced vasoactive substances must be tightly controlled to maintain healthy vascular tone and blood flow in the brain.

Capillary CBF

Although the endothelium of arteries and arterioles predominately controls CBF by releasing vasoactive mediators, the role of endothelial cells in controlling capillary blood flow in the absence of smooth muscle coverage is not as clearly defined. Yet, capillary blood flow is precisely regulated to selectively control CBF in distinct regions to match local tissue O₂ supply and cerebral demand.³³ In fact, accumulating evidence points to the capillaries as major sites of active CBF regulation.^{34,35} Up to 84% of blood flow modulation is estimated to take place at the capillary level.³⁶ Significant heterogeneity exists for blood flow velocities across the capillary bed, as indicated by brain imaging.³⁷ Recent work has elegantly revealed that distributions of microvascular blood flow and oxygenation are layer-specific to facilitate oxygen delivery into the deeper cortical layers,³⁸ illuminating how precisely blood flow is regulated at the capillary level.

The brain capillary bed possesses the highest flow resistance within the cerebral vasculature, which is carefully tuned for proper allocation of blood to all regions. Although the precise way capillary endothelial cells regulate flow is not well understood, the endothelium is known to comprise various mechanoreceptors that can respond to changes in flow velocity and associated pressures.³⁹ Microvascular endothelial cells have the potential to regulate local CBF by responding to changes in the concentration of agonists through Ca²⁺ signaling. One mechanism to control CBF in the capillaries is endothelial communication to upstream arterioles via inward-rectifier K⁺ channels that cause upstream arteriolar dilation and increase blood flow into the capillary bed.⁴⁰ More recently, phosphoinositide PIP₂, a phospholipid that resides in the inner leaflet of the endothelial plasma membrane, was shown to modulate inward-rectifier K⁺ channels. Degradation of PIP₂ by activation of G_q protein-coupled receptors activates capillary Trpv4 channels and dampens signaling to arterioles, which results in altered blood flow.⁴¹ This indicates that the cerebral capillary endothelial cells are electrically connected and coupled to the arteriole smooth muscle compartment to regulate CBF.

Cerebral capillary tubes are covered by pericytes and astrocyte end feet.⁴² Therefore, brain microvascular endothelial cells are positioned to sense signals from axonal terminals.⁴³ The role of pericyte association in terms of CBF

has been debated over the years, but several lines of research have demonstrated that sensory-evoked dilation in first-order vessels can be explained by signal transmission from pericytes via gap junctions to endothelial cells further up the vascular tree. Multiple reports of communication via gap junction coupling of pericytes and the endothelium point to the importance of contact communication between pericytes and the capillary endothelium to modulate microvascular function.⁴⁴ Although smooth muscle cells exhibit a strong contractile phenotype, pericytes appear to lack contractile functions and instead provide a communication network for coordinating information between the neural and vascular compartments.^{45,46} In addition to direct cell-cell communication, capillary endothelial cells can also detect and respond to changes in the concentration of blood-borne agonists through intracellular Ca²⁺ signaling.

Calcium Signaling

Calcium ions serve as important secondary messengers that regulate endothelial cellular processes. Under physiological conditions, endothelial cells maintain a low cytosolic calcium concentration of approximately 100 nmol/L compared with the extracellular calcium ion concentration, which ranges from 1.5 to 2 mmol/L in the brain.⁴⁷ To maintain Ca²⁺ homeostasis, the endothelium employs a series of ion pumps and exchangers to transport excess calcium ions out of the cell or into intracellular calcium stores.⁴⁸ Ca²⁺ influx across the plasma membrane sustains the Ca²⁺ response to both chemical and mechanical stimuli in cerebral endothelial cells.⁴⁹ Cytoplasmic Ca²⁺ is a key regulator of endothelial function, including the synthesis and release of NO, prostacyclin, and other endothelium-derived factors. Although the precise mechanisms by which extracellular Ca²⁺ ions enter the cerebral endothelium are still emerging, many Ca²⁺ channels have been identified. One Ca²⁺ signaling pathway is store-operated Ca²⁺ entry, in which the release from intracellular Ca²⁺ stores activates further Ca²⁺ influx to refill the stores. Stromal interaction molecules on the endoplasmic reticulum membrane subsequently sense store depletion and form functional complexes with Orai molecules, the pore-forming subunits of Ca²⁺ release-activated Ca²⁺ channels on the cell membrane. Both Orai channels and stromal interaction molecule proteins are required for Ca²⁺ release-activated Ca²⁺ channel activity and are important for sustained Ca²⁺ influx from the extracellular environment.⁵⁰ Agonist-driven NO release by acetylcholine is driven by intracellular Ca²⁺ oscillations in the cerebral endothelium, and these Ca²⁺ oscillations are maintained by store-operated Ca²⁺ entry.⁵¹

Mechanical stimuli, as sensed by the endothelium, can induce an elevation in intracellular Ca²⁺. For example, exposure of endothelial cells to fluid shear stress or stretch evokes both Ca²⁺ discharge from intracellular Ca²⁺ pools and Ca²⁺ inflow through mechanosensitive channels in the

plasma membrane.⁵² Unlike the Ca^{2+} response to extracellular agonists, mechanosensitive Ca^{2+} signals typically exhibit either a transient or an oscillatory pattern,⁵³ and these oscillatory signals often appear to propagate between cells as intercellular Ca^{2+} waves.⁵⁴ Shear stress activation of ion channels may be indirect and sensed by the cytoskeleton, apical glycocalyx, mechanosensitive protein complexes, or G protein—coupled receptors.⁵⁵ The diversity of these mechanosensitive responses highlights the complexity of the endothelial Ca^{2+} signaling.

Local Ca^{2+} entry can occur through nonselective transient receptor potential (TRP) channels, which are important in the cerebral endothelium. TRP channels are a diverse family comprising of six cation-permeable channels that can be activated by a wide range of stimuli.⁵⁶ TRP family members include TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), and TRPA (ankyrin).⁵⁷ The TRP channels function as tetramers and conduct cations in response to local environmental cues. Opening of TRP channels depolarizes the cell membrane and leads to intracellular Na^+ and Ca^{2+} accumulation. TRPA1 has been detected in the cerebral endothelium, where Ca^{2+} -associated influx causes endothelium-dependent smooth muscle cell hyperpolarization and relaxation of cerebral arteries.⁵⁸ Endothelial cells can locally produce reactive oxygen species that trigger Ca^{2+} influx through TRPA1 channels and thereby dilate cerebral arteries.⁵⁹ Notably, TRPA1 channel activity is important in mediating hypoxia-induced dilation during ischemic stroke to enhance collateral blood flow to the affected region and, therefore, is considered a sensor of hypoxia.⁶⁰

Increasingly, TRPV4 is regarded as one of the most important Ca^{2+} entry pathways in the endothelium and a major regulator of CBF.⁶¹ TRPV4 channels are polymodal nonselective cation channels that mediate Ca^{2+} entry in response to stimulus. TRPV4 plays an important role in flow-mediated endothelium-dependent vasodilatation.⁶² Shear stress—induced increase in Ca^{2+} can involve the formation of several epoxyeicosatrienoic acids, which contribute to the direct activation of TRPV4.⁶³ Endothelial shear stress sensation also leads to TRPV4-dependent NO release.⁶² TRPV4 channels are also regulated, in part, by phospholipase A_2 . Interestingly, the phospholipase A_2 —sensitive component of Ca^{2+} influx may be polarized to the basolateral face of endothelial cells,⁶⁴ indicating that polarized Ca^{2+} activity in the endothelium may be an important control phenomenon. TRPV4-mediated Ca^{2+} entry is also necessary for the vasodilatory response to acetylcholine in the cerebral arteries.⁶⁵ Experimental evidence from the microcirculatory compartment indicates the importance of ion channels for the capillary endothelium in modulating flow, primarily at TRPV4 and inward-rectifying K^+ channels.⁴¹ These channels appear to enable capillaries to detect local changes in K^+ concentration during neuronal activity and induce changes in endothelial intracellular Ca^{2+}

to rapidly communicate to upstream arterioles and tune CBF. Targeting Ca^{2+} activity at the endothelial level may therefore provide a therapeutic strategy to restore blood perfusion in cerebral vascular disease.

Mechanotransduction and CBF

Endothelium detects changes in local hemodynamic forces. Shear forces from blood flow have been long known to impact the function and health of vascular networks. Decades of research provide evidence for a variety of mechanotransducers on endothelial cells to convert mechanical forces into biological responses. As previously discussed, shear stress sensing by mechanosensitive ion channels on the endothelial plasma membrane can lead to Ca^{2+} influx to drive vasomotor responses. Additional endothelial mechanotransducers include focal adhesions, integrins, primary cilia, the glycocalyx, tight junctions, and adherens junctions.⁶⁶ Because the brain is highly dependent on a continuous blood supply, defective endothelial mechanotransduction can reduce CBF enough to cause ischemic injury. The precise mechanisms by which physical forces are transduced by the endothelium are still emerging, but it is generally accepted that shear stress—mediated forces are transmitted from the apical surface of endothelial cells by the cytoskeleton to sites of cell-cell and cell-matrix interactions.⁶⁷ In cases of obstructive disease such as stroke, collateral vessels can arise to stabilize CBF.⁶⁸ Electron and light microscopy imaging of the mouse brain revealed morphologic differences between endothelial cells residing in the collateral vessels compared with distal-most arterioles. Endothelial cells in the collateral vessels are morphologically aligned with flow and have a continuous layer of smooth muscle cells. An interesting difference between the endothelial cells of the collaterals and arterioles is a reduced number of primary cilia, which are known shear stress sensors, in the endothelium of collateral vessels, where tortuosity is present.⁶⁹ These differences imply that the type of blood flow affects cerebral endothelial cell phenotype and suggest that multiple flow sensors must be important to detect and discern local flow patterns.

Endothelial cells sense the type of local blood flow and remodel their cytoskeleton in response. Cytoskeletal reorganization impacts cell-matrix traction forces as well as cell-cell protein interactions and junctional integrity of the endothelial monolayer.⁷⁰ Flow forces activate endothelial cell surface proteins and adhesive domains that link cytoskeletal proteins and integrins to the underlying matrix. Blocking endothelial adhesion via $\beta 1$ -integrin results in altered cytoskeletal conformation, reorganization of tight junction proteins, and increased vessel permeability.⁷¹ As seen in *in vitro* experiments using human brain microvascular endothelial cells, when cultured under shear stress conditions, endothelial cells up-regulate tight junction protein zonula occludens protein-1 and have decreased

monolayer permeability compared with static counterparts.⁷² The application of shear stress also specifically up-regulates genes that promote drug transporters, ion channels, and junctional proteins, including zonula occludens protein-1 and zonula occludens protein-2.⁷³ Ultimately, data support the idea that blood flow forces strengthen cerebral endothelial tight junctions to help maintain the integrity of the BBB.

Blood-Brain Barrier

The vessels of the brain possess unique properties, termed the BBB, that enable tight regulation of ions, molecules, and cells between the blood and the surrounding brain tissue. The foremost role of the BBB is to maintain homeostasis for optimal brain function, and the endothelium tightly regulates this exchange across the BBB.⁷⁴ Therefore, junctional integrity of the endothelium is essential for the BBB, and impaired CBF has detrimental effects, including increased vessel permeability. In the brain vasculature, endothelial cells are connected by tight junctions to restrict paracellular movement of substances. The major transmembrane proteins in tight junctions include occludins and claudins.⁷⁵ Another type of transmembrane tight junction proteins are the junctional adhesion molecules (JAMs), which regulate immune cell adhesion and transmigration. A decrease of JAM-A occurs in conjunction with BBB breakdown, supporting the idea that endothelial JAM-A contributes to tight junction integrity.⁷⁶ Recently, it was found that JAM-A acts through activation of the cAMP-regulated guanine nucleotide exchange factor I to up-regulate the expression of claudin-5 and limit vascular permeability.⁷⁷ In the setting of cerebral inflammation, JAM-A relocates to the endothelial apical membrane and promotes leukocyte adhesion.⁷⁸ Endothelial cells also express adherens junctions that contain transmembrane proteins, cadherins, of which VE-cadherin is most important for adhesion between cells. Catenins are scaffolding proteins that regulate outside-in signaling, and when bound to cadherin, are stabilized and retained at the cell membrane. *In vivo* data indicates the importance of endothelial-specific stabilization of β -catenin to enhance cerebral barrier maturation.⁷⁹ These experimental findings may open new therapeutic avenues to enhance BBB in the setting of disease.

Also present in cerebral endothelium, gap junctions are primarily formed by members of the connexin and pannexin families. At the plasma membrane, connexins can exist as hemichannels or as gap junctions involved in signaling.⁸⁰ These junctional proteins contribute to the BBB as they regulate permeability, alter transcellular exchange, and modify endothelial metabolic processes. In the setting of acute stroke, the opening of pannexin 1/connexin 43 hemichannels in capillary endothelial cells contributes to the dysregulation of BBB transport functions and mediates the release of ATP.⁸¹ Endothelial cells are also electrically and

metabolically coupled to each other via their gap junctions. This rapid signaling pathway provides coordination of blood vessel function across the cerebral vascular bed.⁸² Hence, disruption of the gap junction signaling between endothelial cells causes breakdowns in vascular control across the capillary bed and can lead to capillary shunting. See [Figure 1](#) for a graphical summary of the endothelial adhesive proteins that maintain the blood-brain barrier.

A unique feature of the cerebral endothelium is the presence of various transport mechanisms to move nutrients in and waste products out of the brain. One such transporter is the glucose transporter isoform 1 that is highly expressed in cerebral endothelial cells and is important for the maintenance of BBB.⁸³ Endothelial cells express transport proteins for a variety of solutes and nutrients, thereby mediating flux in and out of the brain. ATP-binding cassette (ABC) transporters are localized on the surface of endothelial cells and transport a range of organic molecules, cell metabolites, and nutrients. Important for preventing lipid overload in the brain, ABCA1 is expressed in capillary endothelial cells to ensure proper transport of cholesterol.⁸⁴ One of the most studied, ABCB1 has been implicated in barrier function integrity and may have clinical relevance to control drug delivery to the brain. Intriguingly, some transport proteins are polarized in their expression and are expressed at either the luminal or abluminal endothelial cell membrane.⁸⁵ For example, ABCB1 and ABCC5 are expressed on the luminal side, ABCC1 is found primarily on the abluminal membrane, and ABCC2 is found on both sides.⁸⁶ This asymmetric distribution of subcellular localization implies directional transport, and the asymmetry is likely to enhance preferential transport in or out of the vessel.

Inflammation and the Blood-Brain Barrier

Cerebral endothelial cells exhibit a protective phenotype to prevent diffusion of circulating toxins or pathogens. This protective phenotype can be modulated by biochemical stimuli, such as IL-1 α , IL-1 β , and IL-6. Recent work shows that blocking the endothelial IL-1 receptor improves outcomes after ischemic stroke, and therefore, can have therapeutic benefits for cerebrovascular disease.⁸⁷ Alterations in blood flow can also promote the release of cytokines that aggravate the endothelium and induce vessel permeability. Loss of the endothelial protective phenotype compromises the BBB and allows immune cells, inflammatory molecules, and albumin to infiltrate, leading to further complications. These inflammatory agents increase endothelial monolayer permeability and result in leakiness across the vessel wall.

The up-regulation of inflammatory mediators drives cellular damage and can lead to neuronal cell death. The blood-borne agent bradykinin causes BBB permeability by acting on B2 receptors on endothelial cells. The resulting events include elevation of Ca²⁺ and lead to the down-regulation of claudin-5 and increased vessel

Figure 1 Adhesive proteins of the blood-brain barrier. Multiple proteins sustain the integrity of the blood-brain barrier in the cerebral endothelium. Shear stress maintains junctional integrity through the up-regulation of genes that promote the expression of junctional proteins. Capillary endothelial cells (ECs) are joined by tight junctions, gap junctions, and adherens junctions to form the blood-brain barrier. Tight junction proteins include claudins, occludins, and junctional adhesion molecules (JAMs). Gap junctions are composed of the connexin and pannexin families. Adherens junctions contain the transmembrane protein VE-cadherin and associated scaffolding protein β -catenin, which links the cell-cell junctions to the underlying actin cytoskeleton. ZO, zonula occludens protein.

permeability.⁸⁸ The neurotransmitter histamine has been shown to increase BBB dysfunction.⁸⁹ On injury or trauma, the acute release of histamine induces an immediate BBB breach. Cerebral endothelial cells also express the purinergic G protein–coupled receptor P2Y that is activated by extracellular nucleotides, such as ATP and UTP, to modulate BBB permeability.⁹⁰ Further research is needed to uncover the mechanisms that fine-tune the BBB to enable therapeutic treatments to suppress vascular permeability in the setting of cerebral edema or inflammation.

In the setting of inflammation, multiple signaling pathways up-regulate cell adhesion molecules on endothelial cells that trap circulating leukocytes. Adhering leukocytes can plug the vessel and release vasoactive substances or cytotoxic free radicals that cause further endothelial dysfunction. Immune and endothelial cell activation in the capillaries can disturb capillary flow patterns. High-resolution imaging of the ultrastructure of the capillary endothelial cells indicate a higher density of glycocalyx, a carbohydrate-enriched plasma membrane coating, on cerebral endothelium than is present in other capillary beds.⁹¹ This dense endothelial glycocalyx may provide added protection and function as a critical component of the BBB to exert flow resistance in the cerebral capillaries and maintain the BBB.⁹² Recent work shows that degradation of the endothelial glycocalyx by hyaluronidase is associated with higher BBB permeability and exacerbated brain edema in a rat model of asphyxia-induced cardiac arrest.⁹³ In addition, compromised endothelial glycocalyx is associated with endothelial dysfunction in patients with lacunar stroke.⁹⁴ *In vivo* imaging recently revealed that cerebral endothelial cells mediate neurovascular coupling via signaling at caveolae, small invaginations in the plasma membrane

associated with lipid rafts, to control CBF.⁹⁵ Another example of cell surface signaling, the endothelial transmembrane protein sphingosine 1-phosphate receptor-1, was shown to preserve BBB integrity and improve collateral blood flow in a mouse ischemia model.⁹⁶ Intriguingly, sphingosine 1-phosphate receptor-1 was previously shown to protect the endothelial glycocalyx by inhibiting proteinase activity and preserving the heparan sulfate proteoglycan syndecan-1, an integral membrane protein.⁹⁷ These findings point to an important role of endothelial cell surface features in modulating vessel health, and future work may uncover how glycocalyx degradation leads to BBB breakdown.

Vascular Permeability in CSVD

CSVD can lead to reduced cerebral blood flow and increased BBB permeability.⁹⁸ In fact, increased BBB permeability may be an early indicator of multiple forms of CSVD. Magnetic resonance imaging of the brain shows the association between BBB permeability and CSVD in the form of white matter hyperintensities or lesions in patients.⁹⁹ In another study, endothelial dysfunction and BBB integrity were found to be associated with the severity of the lesion.¹⁰⁰ Increased BBB permeability can also lead to stroke.¹⁰¹ Mounting evidence has emerged indicating that circulating markers of endothelial dysfunction may hold promise for early identification of vessel disease. Therapeutic strategies that directly target and reverse endothelial dysfunction can treat CSVD.¹⁰² Higher expression of endothelial inflammatory markers is also associated with white matter hyperintensity progression, further supporting the role of endothelial dysfunction in disease

advancement.¹⁰³ Cerebral vascular permeability and compromised BBB leads to the progression of CSVD.

Although reduced cerebral blood flow is associated with normal aging, more severe CBF reductions have been detected in individuals at risk of developing Alzheimer disease. A study tracking CBF in human subjects carrying the Alzheimer disease genetic risk factor *apolipoprotein E* $\epsilon 4$ allele showed that at-risk individuals exhibited reduced CBF compared with individuals not carrying the allele.¹⁰⁴ Impaired CBF via BBB breakdown was also reported in studies of Alzheimer disease pathogenesis.¹⁰⁵ BBB disruption can lead to an accumulation of macromolecules, such as serum proteins, immunoglobulins, and albumin, that reduce capillary blood flow. High concentrations of thrombin can impair the BBB, which lead to an increase in accumulating neurotoxic molecules and acerbate CSVD.¹⁰⁶ The BBB also regulates the levels of amyloid- β , the main component of amyloid plaques found in

Alzheimer disease. Endothelial cells express the receptor for advanced glycation end products (RAGE) that is implicated in both BBB breakdown and disease progression by promoting amyloid- β influx into the vessel.¹⁰⁷ Accumulation of amyloid- β leads to the up-regulation of endothelial RAGE, reduction in CBF, and continued CSVD progression.¹⁰⁸ Experimentally, the administration of anti-RAGE IgG prevented a reduction in CBF, indicating that RAGE may be a therapeutic target.¹⁰⁹ Besides facilitating the entry of amyloid- β , studies also implicate RAGE in CSVD progression because of the generation of proinflammatory cytokines and ET-1, which cause vasoconstriction and further reduction in CBF.

Progression of CSVD can result in stroke, where brain tissue is damaged because of an acute obstruction or lack of oxygen. Approximately one-fifth of strokes are lacunar strokes, which occur in the deep area of the brain and are associated with CSVD.¹¹⁰ The event of ischemia/

Figure 2 Characteristics of the endothelium in health and disease. With the onset of pathologic conditions, endothelial cells (ECs) undergo compensatory actions in response to circulating immune cells, blood-borne agents (ie, bradykinin and histamine), inflammatory cytokines (ie, IL), and macromolecules (ie, amyloid- β). Endothelial cells up-regulate the expression of surface adhesion proteins intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) that enable binding of immune cells and further promote endothelial activation. Endothelial cells also express the receptor for advanced glycation end products (RAGE), a member of the immunoglobulin superfamily of cell surface molecules, that promotes the entry of amyloid- β . At the intracellular level, cyclooxygenase (COX)-2 sustains an inflammatory state by the release of prostaglandin E2 (PGE2) and reactive oxygen species (ROS). In pathologic conditions, endothelium-derived hyperpolarizing factor (EDHF) induces relaxation of the smooth muscle, whereas endothelin-1 (ET-1) promotes vasoconstriction through binding to endothelin receptor type A (ET_A) receptors on the vascular smooth muscle cell (VSMC). Conversely, ET-1 can induce smooth muscle relaxation via binding on endothelin receptor type B (ET_B) receptors on the endothelium. In a healthy state, endothelial nitric oxide synthase (eNOS) activity is in part regulated by blood flow forces and Ca²⁺ activity to produce the important vasodilator nitric oxide (NO) and ensure proper vascular tone. Mechanisms that protect the endothelium include mechanosensitive ion channels [ie, transient receptor potential vanilloid (TRPV) 4], transporters of cell metabolites and nutrients [ie, ATP-binding cassette (ABC) transporters], cell surface mechanotransducers (ie, primary cilia), tight junctions, and the protective coating of the endothelial glycocalyx. Loss of these attributes, as indicated by the **dashed line**, can lead to endothelial dysfunction and the evolution of cerebral small vessel disease.

Table 1 Markers of Cerebral Endothelial Dysfunction and Associated Pathologic Consequences

Endothelial dysfunction	Pathologic consequences
Reduced nitric oxide production	<ul style="list-style-type: none"> • Impaired vasodilation • Vascular permeability • Increased reactive oxygen species • Reduced CBF
Reduced expression of junctional proteins	<ul style="list-style-type: none"> • Decreased BBB integrity • Increased passage of solutes across vessel wall • Promotes immune cell transmigration
Glycocalyx degradation	<ul style="list-style-type: none"> • Reduced flow resistance • Vascular permeability
Increased expression of adhesion molecules	<ul style="list-style-type: none"> • Immune cell binding • Loss of BBB integrity • Vascular inflammation
Increased proliferation	<ul style="list-style-type: none"> • Loss of vessel homeostasis • Enables uncontrolled angiogenesis
Increased secreted factors	<ul style="list-style-type: none"> • Vascular permeability • Promotes vasoconstriction • Vessel damage • Altered CBF

BBB, blood-brain barrier; CBF, cerebral blood flow.

reperfusion after stroke is known to initiate an inflammatory cascade and increase BBB permeability as circulating immune cells migrate toward the ischemic lesion to release inflammatory cytokines and activate the endothelium.¹¹¹ Leukocytes adhere to the endothelium and aggravate reperfusion injury by releasing tumor necrosis factor- α and IL-1, which mediate endothelial inflammatory activation. Endothelial cells up-regulate the expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, which then promote the adhesion and transendothelial migration of leukocytes.¹¹² In particular, increased expression of intercellular adhesion molecule-1 on endothelial cells facilitates the transendothelial migration of leukocytes. The efficacy of intercellular adhesion molecule-1 inhibition to protect against ischemic injury, however, has had mixed outcomes. Although the application of intercellular adhesion molecule-1 blocking antibodies reduced brain injury in a model of cerebral ischemia,¹¹³ transgenic mice deficient in *Icam1* failed to reduce ischemia-induced accumulation of neutrophils in the brain.¹¹⁴ miRNAs may improve BBB dysfunction by targeting specific Ly6C^{High} proinflammatory immune cells to preserve cerebral endothelial cell function.¹¹⁵ Another miRNA, the miR-15a/16 to 1 cluster, acts as a negative regulator for postischemic angiogenesis by repressing the expression of pro-angiogenic factors.¹¹⁶ Future efforts that target endothelial miRNA function may prove to be a viable therapeutic approach for normalizing cerebral vessel function. Ultimately, BBB dysfunction and associated inflammation contribute to CSVD progression, but continued research is needed to understand the complex

mechanisms of vessel dysfunction and develop effective therapeutics.

Conclusion

Maintenance of CBF requires the dynamic actions and interactions of the cerebral endothelium to affect changes in blood vessel function. Endothelial cells are responsive to changes in their environment, including blood flow forces, cell-cell and cell-matrix interactions, secreted factors, and second messengers. The ability of endothelial cells to generate stimulus-specific responses to these inputs is facilitated by signaling molecules, ion channels, junctional complexes, and surface proteins that enable localized signaling to activate multiple pathways that control vessel function. See [Figure 2](#) for a graphical representation of the cerebral endothelium in both healthy and disease states.

A healthy brain requires constant blood perfusion and control of CBF, and, in the event of flow disturbances, can lead to CSVD. The cerebral endothelium is a key mediator of brain health by keeping CBF within normal physiological limits. Continued research is needed to better define the types of cerebral endothelial dysfunction and how the dysfunction profile links to CSVD pathology. Although [Table 1](#) lists known markers of endothelial dysfunction and the associated pathologic consequences, further refinement of the dysfunction characteristics is necessary to correlate with measurable clinical symptoms of CSVD. Delineation of the endothelial dysfunction profile can facilitate clinical readouts, such as biomarkers, and lead to therapeutic targets. With technological advances in high-resolution optical imaging, biosensors, and single-cell transcriptomics, future studies will help clarify the mechanisms by which the cerebral endothelium modulates CBF and vessel health to enable both early detection of CSVD and effective clinical treatments. Ultimately, early detection and clinical treatment may halt or reverse disease progression to improve and extend patient lives.

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