Chapter 15
Mechanosensitive Calcium Fluxes in the Neurovascular Unit: TRP Channel Regulation of the Blood-Brain Barrier

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Abstract The blood-brain barrier of the neurovascular unit is a critical organ for normal brain function. It is continuously exposed to mechanical stress both from the peripheral circulation and plasma osmolarity changes. Barrier integrity is regulated by a number of mechanisms, including influx of calcium ions and activation of calcium-sensitive signaling pathways. This review addresses the molecular identity of the channels underlying mechanosensitive calcium influx, and hypothesizes a central role for transient receptor potential channels in mechanosensitive regulation of blood-brain barrier endothelial cell function.

Keywords Calcium entry pathways · mechanically gated channels · mechanosensitive channels · voltage-gated channels · purinergic receptors · sodium-calcium exchanger · transient receptor potential channels · calcium-dependent modulation

15.1 Introduction

The neurovascular unit (NVU) is a critical feature of the brain circulatory system, and regulates normal cerebral blood flow and brain function. The NVU (Fig. 15.1) consists of multiple cell types, including endothelial cells, astrocytes, pericytes and neurons (Abbott et al., 2006). In brain capillaries, the NVU is characterized by the presence of the blood-brain barrier (BBB), a structure consisting of tight junctions between capillary endothelial cells (Rubin and Staddon, 1999) and the polarized expression of specific transport systems (Dermietzel and Krause, 1991). The tight junctions between brain capillary endothelial cells are sufficient to impart high electrical resistances across the capillary wall (Rubin and Staddon, 1999), indicating that paracellular diffusion of ions from the blood to the brain is highly restricted. The BBB is critical in

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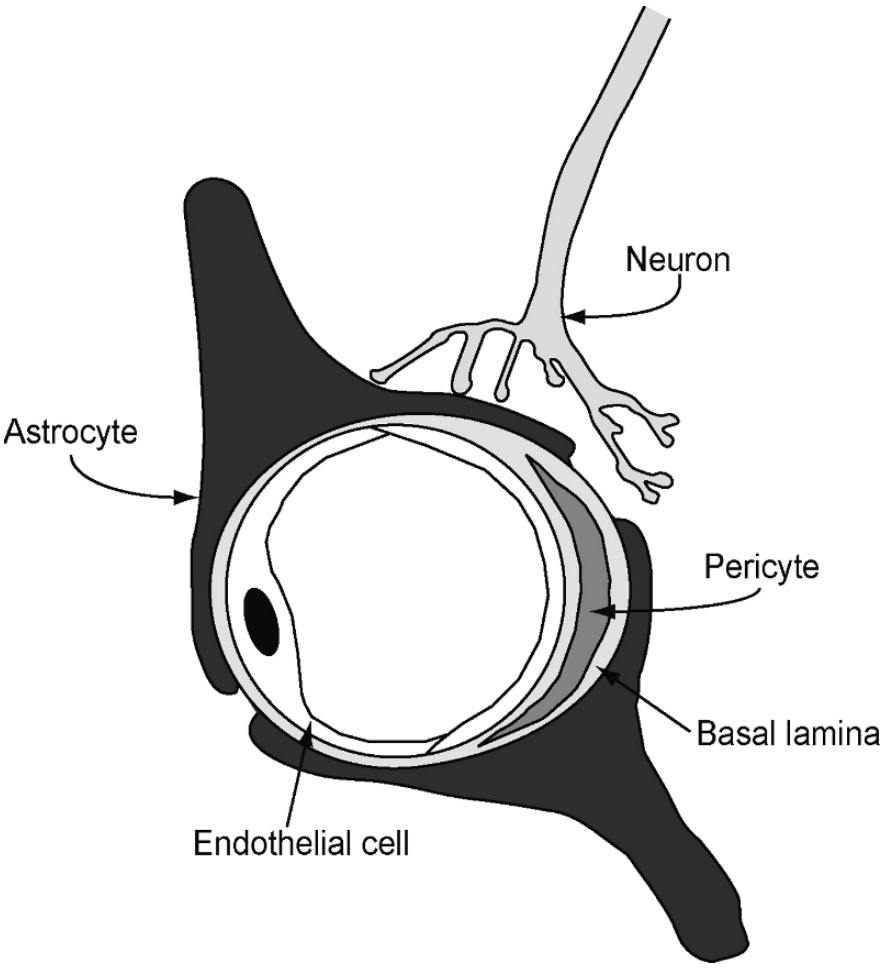

Fig. 15.1 The neurovascular unit and blood-brain barrier. The neurovascular unit consists of multiple cell types. Endothelial cells that form the walls of the brain capillaries, surrounded by the basal lamina, form the walls of the brain capillaries. Astrocytes send foot processes to the capillaries, which wrap around the basal lamina along portions of the capillary. Neuronal processes also extend to the capillaries. Finally, pericytes may be found within the basal lamina, where they can interact with capillary endothelial cells

regulating macromolecular and ionic gradients within the brain, allowing for normal neuronal activity.

The integrity of the NVU/BBB is compromised in numerous pathological conditions, including Alzheimer's disease, multiple sclerosis, inflammation, diabetes and stroke (Ballabh et al., 2004; Hawkins and Davis, 2005). These disruptions allow for the diffusion of proteins, ions, water and other compounds from the blood into the brain. Many disruptive stimuli share a common

cellular event within the endothelial cells: an increase in the level of intracellular calcium and the activation of calcium signaling cascades (Aschner et al., 1997; Nagashima et al., 1997; Bartha et al., 2000; Brown and Davis, 2002; Easton and Abbott, 2002). Prevention of calcium influx can protect the BBB in a number of scenarios (Abbruscato and Davis, 1999; Easton and Abbott, 2002), implicating calcium ions as critical regulators of BBB structure and function (Brown and Davis, 2002).

For some disrupting stimuli, such as stroke, the dysregulation of plasma macromolecule and ion fluxes across the capillary walls in the infracted area contributes to the osmotic movement of water into the brain, resulting in edema formation (Petty and Wettstein, 2001). This edema contributes to secondary injury to neurons. While many studies have addressed the role of hypoxic stress in disrupting the BBB (Plateel et al., 1997; Abbruscato and Davis, 1999; Fischer et al., 2002; Mark and Davis, 2002; Brown et al., 2003; Fischer et al., 2004; Mark et al., 2004; Krizbai et al., 2005), the role of mechanical stress associated with a loss or reduction in blood flow and shear stress as a potential contributing factor to NVU endothelial cell dysfunction in stroke is not fully appreciated. In this review we will discuss the potential role of mechanical stresses and their alteration in the regulation of the NVU, particularly the altered mechanical stress experienced by NVU endothelial cells in response to changes in blood flow and blood osmolarity.

15.2 Calcium Entry Pathways: A Mechanosensitive Link

As previously mentioned, many stimuli that disrupt the BBB cause an increase in intracellular calcium, including thrombin (Lerner, 1994; Bartha et al., 2000; Kim et al., 2004), histamine (Li et al., 1999; Paemeleire et al., 1999; Paltauf-Doburzynska et al., 2000), bradykinin (Paemeleire et al., 1999; Easton and Abbott, 2002), ATP (Tanaka et al., 2004), and exposure to hypoxic stress (Kimura et al., 2000; Brown et al., 2004b). Blocking elevation of intracellular calcium prevents BBB disruption by many of these treatments (Li et al., 1999; Bartha et al., 2000; Brown et al., 2004b; Tanaka et al., 2004; Brown et al., 2008). However, the underlying mechanisms of calcium entry, and the subsequent events in NVU endothelial cells, are not well understood, especially for mechanical stimuli.

15.2.1 Voltage-Gated Channels

There are a number of potential protein candidates for calcium entry channels in NVU endothelial cells. Pharmacological evidence exists for the presence of L-type calcium channels in these cells (Abbruscato and Davis, 1999; Berkels et al., 1999; Yakubu and Leffler, 2002), with L-type calcium channel blockers

protecting against barrier disruption. However, there is little functional evidence for voltage-gated calcium channels in these non-excitable cells, and no convincing molecular evidence for the expression of L-type calcium channels in NVU endothelial cells. Furthermore, there is evidence of off-target effects of L-type channel blockers on calcium fluxes (Berkels et al., 1999; Hempel et al., 1999), and they are not effective at preventing intracellular calcium increases or barrier disruption (Stanimirovic et al., 1994; Ikeda et al., 1997; Wei et al., 2004). Hence a role for voltage-gated channels in NVU mechanosensitive phenomena is not anticipated. However, L-type calcium channels are present on the other cells of the NVU, including astrocytes, pericytes and neurons (Barres et al., 1989; Catterall, 1998; Westenbroek et al., 1998; Kamouchi et al., 2004), and may be important in mediating calcium influx in those cell types.

15.2.2 Purinergic Receptors

Purinergic receptors are expressed on NVU endothelial cells, specifically P2Y receptors (Nobles et al., 1995; Sipos et al., 2000). Other endothelial cells express P2U receptors (Miyagi et al., 1996) and P2X receptors (Ramirez and Kunze, 2002; Harrington et al., 2007), but these have not been found at the BBB. P2Y receptors are G protein-coupled receptors (GPCR) (Burnstock, 2006), while P2X receptors are calcium-permeable ligand-gated ion channels. Activation of P2Y receptors increases intracellular calcium levels in endothelial cells by inducing release of calcium from intracellular storage sites (Albert et al., 1997; Duchene and Takeda, 1997), and can mediate alterations in vascular endothelial cell monolayer permeability (Tanaka et al., 2004). However, since P2Y receptors are GPCR, these signaling receptors do not directly mediate calcium influx at the BBB.

15.2.3 The Sodium-Calcium Exchanger

A striking characteristic of NVU endothelial cells is the high level of polarized expression of numerous transporters, which allow for CNS penetration of ions, peptides and drugs (Keep et al., 1993; O'Donnell et al., 1995; Vannucci et al., 1997; Jolliet-Riant and Tillement, 1999; Chishty et al., 2003). A major transporter involved in calcium flux at the BBB is the sodium-calcium exchanger (NCX) (Sedova and Blatter, 1999). Under normal physiological conditions, the NCX transports calcium out of the cells in exchange for sodium, maintaining a low intracellular calcium concentration. However, under stroke-like conditions, with elevated intracellular sodium levels, the NCX can reverse (Li et al., 2000; Berna et al., 2001), potentially contributing to calcium influx and activation of calcium signaling cascades and subsequent downstream cellular responses.

15.2.4 Transient Receptor Potential Channels

Recent studies in our laboratory have focused on the transient receptor potential (TRP) channel superfamily as candidates for mediating calcium influx in NVU endothelial cells. There are currently seven TRP families, and the channels can be activated by many different stimuli (Venkatachalam and Montell, 2007). Several of these families encode cation permeable channels that mediate calcium flux in a number of cell types (Caterina et al., 1997; Pizzo et al., 2001; Gao et al., 2003; Nilius et al., 2003; Kraft et al., 2004; White et al., 2006). A number of mammalian TRP channels are characterized as "mechanosensitive", including TRPA1, TRPP, TRPC1, TRPC6, TRPV1, TRPV2 and TRPV4 (Nilius and Voets, 2004; Lin and Corey, 2005; O'Neil and Heller, 2005; Spassova et al., 2006)

The TRPA1 channel is located on the stereocilia in hair cells of the mouse inner ear. siRNA downregulation of protein expression reduces transduction currents in transfected hair cells (Corey et al., 2004). Embryonic expression of TRPA1 is correlated with the onset of mechanosensitivity in hair cells (Corey et al., 2004). The multiple ankyrin repeats of TRPA1 are hypothesized to act as a gating spring, transducing stereocilia bending to channel opening (Sotomayor et al., 2005). However, there is no evidence for TRPA1 expression in vascular endothelial cells in general (Yao and Garland, 2005), or in NVU endothelial cells specifically, although to date the presence of this, and many other TRP channels has not been specifically examined at the BBB.

TRPP channels are associated with polycystic kidney disease (Delmas, 2005). Two proteins in the TRPP family, PKD1 (TRPP1) and PKD2 (TRPP2), have been linked to mechanosensation in primary cilia of kidney tubule epithelial cells (Nauli et al., 2003). Expression of both proteins is required for the expression of functional ion channels (Hanaoka et al., 2000), with TRPP2 being the pore-forming subunit. Knockout of PKD1 eliminates flow-activated calcium influx in kidney epithelial cells (Nauli et al., 2003), indicating activation of the channel by fluid flow/shear stress. PKD1 has been proposed as the flow sensor in the TRPP mechanosensitive channel (Forman et al., 2005). Activation of the channel is thought to occur by actual mechanical deformation of the extracellular domains of PKD1 by fluid flow through the kidney tubule (Forman et al., 2005), and presumably in other TRPP expressing tissues. Like TRPA1, the presence of TRPP channels at the BBB has not yet been described.

TRPC1 has been implicated in mechanosensation to stretch in oocytes (Maroto et al., 2005), but does not appear to be responsible for smooth muscle activation after hypo-osmotic swelling or increases in pressure (Dietrich et al., 2007), indicating that TRPC1 alone may not serve as a mechanosensitive channel in endogenous expression systems. Mechanisms of activation for TRPC1 include store-depletion, (Beech, 2005), and stretch, though the exact process by which stretch can activate TRPC1 is unclear. More recent studies on TRPC1 and TRPC6 have indicated that these channels may not underlie mammalian stretch-activated mechano-sensitive Ca^{++} permeable cation channel

(MscCa) activity (Gottlieb et al., 2007). Our recent work has demonstrated expression of TRPC1 in NVU endothelial cells (Brown et al., 2008), as well as TRPC2, C4 and C7, none of which are implicated in mechanosensation at this time. There is some evidence for the expression of TRPC6 in the NVU (Brown et al., 2008), but it is not clear if this expression is in the endothelial cells or in other cell types.

The TRPV channels are strongly implicated in the transduction of mechanical stress into cellular responses (O'Neil and Heller, 2005). TRPV4 was first identified in the kidney tubule, and mediates responses to hypo-osmolar treatment (Liedtke et al., 2000) and shear stress (Gao et al., 2003; Taniguchi et al., 2007; Wu et al., 2007). TRPV4 knockout mice show impaired responses to heat, osmolarity and pressure (Liedtke and Friedman, 2003; Mizuno et al., 2003; Suzuki et al., 2003; Lee et al., 2005; Levine and Alessandri-Haber, 2007), indicating that TRPV4 serves as an important member of a mechanosensitive complex. TRPV1 and TRPV2 are also implicated in transducing mechanically-induced signals (Birder et al., 2002; Muraki et al., 2003). Our data indicates that NVU endothelial cells express TRPV2 and TRPV4 (Brown et al., 2008), both of which potentially mediate mechanical signal transduction at the BBB.

In summary, although there are a number of transport proteins that might mediate calcium influx into NVU endothelial cells, the TRP channels are the best candidates for transducing mechanical signals. Of the multiple TRP isoforms that are mechanosensitive, expression of TRPC1, TRPV2 and TRPV4 has been demonstrated at the BBB (Brown et al., 2008). In particular, since TRPV4 has been shown to be sensitive to hypotonic cell swelling in aortic endothelial cells (Vriens et al., 2005), to flow in mid-cerebral artery endothelial cells (Kohler et al., 2006; Marrelli et al., 2007) and to both flow and hypotonic cell swelling in renal collecting cells (Gao et al., 2003; Wu et al., 2007), it is likely that the channel also plays a significant role in mechanosensitive properties of the BBB.

15.3 Calcium-Dependent Modulation of BBB Integrity

A common event in the disruption of the BBB by various stimuli is an increase in intracellular calcium (Greenwood, 1991; Nagashima et al., 1997; Abbruscato and Davis, 1999; Paemeleire et al., 1999; Bartha et al., 2000), leading to subsequent loosening of BBB tight junctions and increased barrier permeability. We hypothesize two mechanisms by which calcium ions regulate barrier function: (1) interaction of calcium ions with the proteins of tight and adherens junctions, either intracellularly at the site of the junctional complex, or in the extracellular space (Fig. 15.2), and (2) modulation of calcium-sensitive signaling pathways that can alter protein phosphorylation, cell-cell adhesion and barrier function.

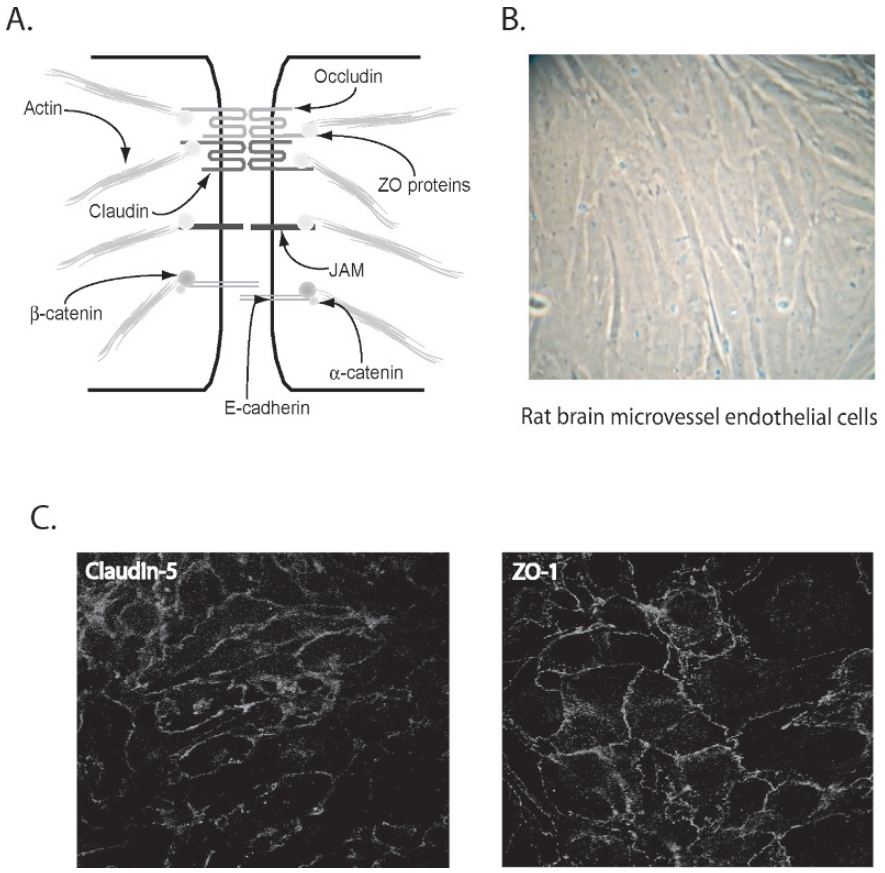

Fig. 15.2 Tight junctions in BBB endothelial cells.
The tightness of the BBB is due to the presence of tight junctions between adjacent endothelial cells. (**A**) The tight junction, at the apical or blood side of the brain capillary endothelial cell, consists of multiple transmembrane proteins (occludin and members of the claudin family) that are anchored to the actin cytoskeleton by the zonula occludens (ZO) proteins. Adherens junctions are present towards the basolateral, or brain, side of the capillary wall. Another contributor to cell-cell adhesion is the JAM family of proteins. (**B**) Rat brain microvessel endothelial cells in culture form characteristic spindle shapes and are closely packed. (**C**) Confocal microscopy staining for two tight junction proteins in an immortalized mouse brain endothelial cell line. Both claudin-5 and ZO-1 are closely aligned with the cell membrane, indicating their localization in tight junctions

15.3.1 Protein-Calcium Ion Interactions

There is certainly evidence of a direct role for calcium in the maintenance of adherens junctions between adjacent cells (Gumbiner and Simons, 1987; Gumbiner et al., 1988; Bazzoni and Dejana, 2004), but this is directly linked to

extracellular, not intracellular calcium levels. Chronic depletion of extracellular calcium can also disrupt localization of zonula occludens-1 (ZO-1) in the tight junction (Riesen et al., 2002), but the time course of this response does not allow for differentiating between direct ion interactions and signaling cascade actions of increased intracellular calcium. There is evidence that low intracellular calcium levels interfere with tight junction assembly and function (Stuart et al., 1994; Ye et al., 1999), but again, there is no evidence that this is due to direct interactions of calcium ions with tight junction proteins. To date, the literature does not suggest a direct role for calcium in regulating BBB tight junctions and barrier function, such as that seen with adherens junctions. However, modulation of adherens junctions can also contribute to BBB leakiness (Tiruppathi et al., 2002).

15.3.2 Calcium-Dependent Signaling Pathways

While a direct role for calcium in modulating BBB function is not currently supported by the literature, there is ample evidence for an indirect role for calcium in mediating barrier function via calcium sensitive signaling cascades. Endothelial barrier disruption by inflammatory mediators, such as thrombin and histamine, can occur via calcium-induced activation of myosin light chain kinase (MLCK) and dissociation of adherens junctions (Tiruppathi et al., 2002). Studies in lung endothelial barriers indicate that thrombin exerts its disruptive effects via calcium-calmodulin kinase II (CaMKII) and the ERK signaling pathway (Borbiev et al., 2001; Borbiev et al., 2003). This leads to subsequent activation of MLCK and contraction of the cytoskeleton (Gunduz et al., 2003), pulling adjacent endothelial cells apart. This signaling pathway also involves G_o linked GPCRs (Vanhauwe et al., 2002) and phospholipase C (Kim et al., 2004). Thrombin-induced calcium influx has been linked to TRPC1 (Paria et al., 2004), and thrombin-induced phosphorylation of TRPC1 via protein kinase C is important for TRPC1 activation and calcium influx (Ahmmed et al., 2004).

While the CaMKII-ERK-MLCK pathway for barrier disruption has been demonstrated extensively in endothelial cell systems, it remains to be seen if these players mediate BBB function in a similar fashion. Activation of MLCK could explain the morphological changes seen in NVU endothelial cells after exposure to numerous disrupting stimuli, but this has not been extensively studied as of yet (Haorah et al., 2005). Furthermore, there are other calcium-sensitive signaling mechanisms that may contribute to BBB disruption, including calcium-calmodulin regulated production of nitric oxide (Busse and Mulsch, 1990; Thiel and Audus, 2001; Mark et al., 2004), activation of PKC regulated signaling pathways (Grammas et al., 1998; Fischer et al., 2004; Fleegal et al., 2005), and calcium-regulated phosphorylation of tight junction proteins (Andreeva et al., 2001; Ishizaki et al., 2003; Kale et al., 2003; Ohtake et al., 2003).

15.4 Sensing Mechanical Stress at the NVU

Endothelial cells of the vasculature are highly sensitive to mechanical forces relating to pressure/stretch (biaxial stress) and to shear stress arising from blood flow (Nilius et al., 2003; Busse and Fleming, 2006). Accumulating evidence points to a likely role of TRP channels in transducing mechanical signals in NVU endothelial cells, although our understanding of the extent of the stresses, and the sensing and transduction of these stimuli into cellular responses at the NVU is still forthcoming. There are a number of pathological scenarios in which this signaling may play a role in normal CNS function. Two of the most common scenarios are discussed below: changes in shear stress/blood flow and/or blood pressure due to stroke or hypertension, and changes in plasma osmolarity, leading to cell volume changes and cell membrane stress, as occurs in hyponatremia (hypo-tonicity) or induced hyperosmolar BBB disruption to treat brain tumors. The glycocalyx appears to play a central role in sensing mechanical stresses in vascular systems and will be considered first.

15.4.1 Role of the Glycocalyx in Sensing Mechanical Stress

Any discussion of endothelial cell response to mechanical stress must include consideration of the glycocalyx. The glycocalyx is a thin network of glycoproteins lining all blood vessels. It consists of membrane-bound molecules, including sulfated proteoglycans, hyaluronan, glycoproteins and plasma proteins (Weinbaum et al., 2007). The endothelial glycocalyx is negatively charged, and typically includes glycoproteins with acidic oligosaccharides and sialic acid, as well as proteoglycans with glycosaminoglycan side chains, including heparan sulfate, chondroitin sulfate and hyaluronan (Tarbell and Pahakis, 2006; Weinbaum et al., 2007). The glycocalyx is critical to the transduction of shear stress forces to the endothelial cells, and their subsequent response to this stress. Current theories indicate that the glycocalyx mediates shear stress by transducing flow to the endothelial cells via the membrane-bound glycocalyx proteins (Tarbell and Pahakis, 2006); the endothelial cell membrane may not experience shear stress directly. The structure and function of the glycocalyx is dependent on hydration (Weinbaum et al., 2007), and can be disrupted by inflammatory mediators (Henry and Duling, 2000) or by enzymatic digestion (Vogel et al., 2000).

There have been a number of studies examining the BBB glycocalyx and its composition. Initial studies indicated no difference in glycocalyx composition between brain, retina and myocardium (Lawrenson et al., 2000), or between in vivo vessels and in vitro cultures (Fatehi et al., 1987). Removing the glycocalyx with heparinase can increase perfusion and cerebral blood flow (Vogel et al., 2000). Because of the net negative charge of the

glycocalyx, permeability of the BBB to anionic compounds is lower then the permeability to neutral compounds, particularly at smaller sizes (Sahagun et al., 1990).

An emerging model for transduction of shear stress through the glycocalyx is dependent on membrane-bound glycoproteins, including CD44 and syndecans, which transmit shear stress through the glycocalyx layer to the cell membrane (Weinbaum et al., 2007). These transducing molecules are linked to the cytoskeleton, and movement of the outer portions of the proteins in response to blood flow is conducted to the cytoskeleton through the membrane glycoproteins. The plasma membrane of the endothelial cells may not experience any shear stress directly. The subsequent consequences of the transmission of the shear stress are not well understood, but may include activation of mechanosensitive ion channels in the cell membrane, especially glycosylated channels, where the glycosylated moieties may protrude into and be a part of the glycocalyx structure.

15.4.2 Sensing Changes in Shear Stress/Blood Flow at the NVU

Shear stress attributed to blood flow has great effects on NVU endothelial cells. In culture, brain endothelial cells tend to grow in a disorganized monolayer, with no orientation to the spindle shaped cell bodies (Fig. 15.2). However, if brain endothelial cells are grown under conditions of flow, they orient parallel to the direction of the flow (Neuhaus et al., 2006) and develop higher transendothelial electrical resistance (TEER) then cells grown without flow (Stanness et al., 1997). Shear stress also induces lamellopodia formation and focal adhesion formation in endothelial cells in culture, shifting actin and vimentin stress fibers (Mott and Helmke, 2007). Shear stress inside the capillaries can change in two ways: a decrease, such as in a stroke, or an increase, as in hypertension.

15.4.2.1 Decreased Blood Flow – Stroke

Ischemia and reperfusion are known to damage the glycocalyx, altering the mechanical forces on the endothelial cells. This response can be prevented by ischemic preconditioning, and partially blocked by treatment with superoxide dismutase, indicating a role for reactive oxygen species in glycocalyx disruption (Beresewicz et al., 1998). This damage typically occurs during reperfusion, when shear stress goes from very low back to normal. The BBB is also disrupted during ischemia/reperfusion injury in vivo (Kuroiwa et al., 1988; Preston and Webster, 2002), although the endothelial cells themselves seem to recover during reoxygenation in an in vitro model (Mark et al., 2001). It is difficult to tease apart the relative contributions of hypoxic and shear stress to BBB dysfunction in animal models. An *in vivo* study by Hom and coworkers in 2001 demonstrated little or no BBB disruption with lowered perfusion pressure for a 20 min

in situ perfusion study (Hom et al., 2001). Similarly, a study of BBB endothelial cells grown in capillary flow systems demonstrated no alteration of BBB permeability after transient loss of flow for one hour (Krizanac-Bengez et al., 2003). However, there have only been a few studies on loss of flow in shear stress models, and in all of those the lowered shear stress was only for a brief period of time. Longer exposures to lowered flow may cause alterations in BBB permeability in isolation, or after reperfusion/resumption of normal shear stress in the model system.

15.4.2.2 Increased Blood Flow – Hypertension

Thanks to animal models of hypertension, there is a body of literature examining the effects of increased blood pressure on BBB structure and function. There is a well characterized decline in BBB function with age (Mooradian, 1988), which can be exacerbated by the development of high blood pressure. Several recent studies in the stroke-prone spontaneously hypertensive rat (SPSHR) and the spontaneously hypertensive rat (SHR) have investigated BBB function at increased blood pressures. SHR and SPSHR showed increased vascular permeability to horseradish peroxidase (MW 44 kDa) in the hypothalamus as compared to controls (Ueno et al., 2004). These hypertensive animals also showed disruption of the glycocalyx. High blood pressure is also correlated with increased damage after middle cerebral artery occlusion in SHR (Hom et al., 2007). These studies suggest that hypertension alone disrupts the BBB, and it exacerbates barrier opening after additional ischemic insult. This is consistent with studies in other vascular endothelial cells showing increased calcium influx with increases in shear stress (Nilius et al., 2003).

15.4.2.3 Channels Modulated by Shear Stress/Blood Flow

Stretch or shear stress applied to endothelial cells activates calcium influx as part of the cellular response. While the focus of this review is on the role of TRP channels in brain microvessel endothelial cells, other channels may also be sensitive to mechanical stimulation, including inward-rectifying potassium channels and outward-rectifying chloride channels (Gautam et al., 2006). These channels may play important roles in the vascular response, especially for the larger vessels where the interplay of endothelial cells with the surrounding smooth muscle cells may be critical. Several excellent reviews covering these other channels have been published and will not be discussed here (Coleman et al., 2004; Gautam et al., 2006).

TRP channels are emerging as potentially central players in endothelial responses to shear stress, especially in medium to large vessels (Nilius et al., 2003; Gautam et al., 2006). Several TRP channels are potential candidates for sensing shear stress/pressure in the brain microvessel endothelial cells, as discussed above. Two channels with the strongest tie-in as a sensor, or a

component of a sensor, are TRPV2 and TRPV4, both of which are expressed in brain microvessel endothelial cells (Brown et al., 2008). TRPV4 is sensitive to hypotonic cell swelling in the bEnd3 brain endothelial cell line and has been shown in separate studies to be highly sensitive to increases in shear stress in both overexpression systems and in renal epithelial cells endogenously expressing TRPV4 (Gao et al., 2003; Wu et al., 2007; Brown et al., 2008). Knockdown of TRPV4 in non-NVU cells by siRNA techniques abolishes flow- and hypotonicity-induced calcium influx (Wu et al., 2007). Further, TRPV4 has now been shown to be sensitive to fluid flow in middle cerebral artery endothelial cells (Kohler et al., 2006; Marrelli et al., 2007). Hence, it is likely that the TRPV4 channel also plays a significant role in mechanosensitive properties of the BBB, and it may be a primary source of mechanically-induced calcium influx in these cells; further supporting evidence for this and other TRP channels as mechanosensitive channels at the BBB remains to be fully established.

While TRPV4 may be sensitive to mechanical stimulation, the mechanism of activation is likely to be indirect. The kinetics of channel opening following application of shear stress are relatively slow, requiring many seconds to activate (Wu et al., 2007). Further, Nilius and coworkers demonstrated that the channel could be activated by downstream metabolites of arachidonic acid which were induced by hypotonicity-induced swelling (Vriens et al., 2004). Blockade of arachidonic acid metabolism abolished hypotonicity-induced activation of TRPV4, thereby demonstrating that the activation of TRPV4 by mechanical stimulation by an indirect biochemical pathway. Whether a similar mechanism of activation by shear stress is in play at the NVU seems highly probably, but has not yet been directly demonstrated.

15.4.3 Sensing Changes in Plasma Osmolarity and Cell Volume

Other physiological events can cause mechanical stress on NVU capillary endothelial cells, such as changes in blood osmolarity, which in turns leads to swelling or shrinking of endothelial cells. Clinically, there are a number of situations in which blood osmolarity may be altered; here we will discuss hypo-osmolarity arising from hyponatremia, and hyper-osmolarity after infusion of hyper-osmolar mannitol solutions to aid in the delivery of chemotherapeutics to brain tumors.

15.4.3.1 Hypo-Osmolarity – Hyponatremia

Hyponatremia is a condition in which plasma sodium levels drop below the normal range, due to loss of sodium or retention of water. One cause of hyponatremia in otherwise healthy people occurs in the case of endurance

athletes participating in long events, such as long distance running events (Noakes, 2002) and Ironman triathlons (Speedy et al., 1997), or with water intoxication, such as in patients suffering from psychogenic polydipsia (Dundas et al., 2007). In these cases, the patient loses sodium through sweating and urinary loss or dilutes plasma sodium levels by excessive intake of water. The loss of sodium in athletes can be exacerbated by intake of excessive amounts of water, leading to water intoxication and low plasma sodium levels (Speedy et al., 1997; Noakes, 2002; Noakes, 2003). In some instances, overhydration and hyponatremia can be fatal in otherwise healthy individuals (Garigan and Ristedt, 1999). The greatest risk to individuals with hyponatremia is the development of cerebral edema, leading to disorientation and seizures (Oster and Singer, 1999; Baker et al., 2000).

In normonatremia, blood osmolarity is typically near 290 mOsm/l, whereas hyponatremic osmolarity can drop to 250 mOsm/l (Oster and Singer, 1999). Low plasma osmolarity alone does not seem to disrupt the BBB in the clinical setting, but we have shown that hypotonicity can induce a transient disruption of the BBB *in vitro* (Brown et al., 2008). Instead, it is the rapid correction of plasma sodium levels that causes disruption of the BBB, potentially leading to osmotic demyelination syndrome (Adler et al., 1993; Adler et al., 1995; Baker et al., 2000). This rapid correction can also lead to increased cerebral blood flow (Adler et al., 2000), and allows for the penetration of the brain by inflammatory mediators such as IgG and activated complement C3d (Baker et al., 2000). Furthermore, chronic hyponatremia seems to lead to a lower osmotic threshold for BBB disruption after correction (Adler et al., 1995), indicating that the endothelial cells of the BBB adapt to lowered plasma osmolarity in some fashion that makes them more sensitive to a rapid increase in osmolarity with correction, leading to an increased risk of BBB disruption and edema formation. Whether this is related to activation of hypo-osmotic-sensitive TRP channels, such as TRPV4, is currently not known. NVU endothelial cells express two hypo-osmolar sensing channels, TRPV2 and TRPV4 (Brown et al., 2008).

15.4.3.2 Hyper-Osmolarity – Delivery of Chemotherapeutic Agents to Inoperable Brain Tumors

The ability of hyper-osmolar agents to reversibly open the BBB has been known for almost forty years (Rapoport, 1970; Rapoport et al., 1971). Infusion of hyperosmolar mannitol is used clinically to enhance the delivery of chemotherapeutic agents across the BBB to targeted tumors (Neuwelt et al., 1979; Gumerlock et al., 1992). In animal models, there is regional variation in the degree of barrier disruption in the brain (Brown et al., 2004a), and hypertension alters the time course of barrier opening (Al-Sarraf et al., 2007). Exposure to hyper-osmolar mannitol triggers calcium influx into BBB endothelial cells (Paemeleire et al., 1999) and disrupts tight junction structure (Nagy et al.,

1979). This calcium influx is thought to trigger signaling cascades that result in BBB disruption (Nagashima et al., 1994; Nagashima et al., 1997). However, BBB disruption by hyper-osmolar solutions is transient, and the barrier recovers within a short period of time (around 1 hr) (Brown et al., 2008). This rapid response and subsequent normalization would suggest an acute cellular response involving receptor activation and desensitization rather then global cellular changes involving the expression of new proteins.

The mechanism of the hyper-osmotic barrier disruption and associated calcium influx is currently not defined. Could it involve shrinkage-induced activation of TRP channels? This is an intriguing possibility since a similar scenario may be at play in the osmo-sensitive neurons of the hypothalamus that control vasopressin secretion from the posterior pituitary. Application of hyper-osmotic media to neurosecretory supraoptic nucleus neurons and to the organum vasculosum lamina terminalis neurons have been shown to induce calcium influx in these cells as part of a mammalian osmoreceptor (Ciura and Bourque, 2006; Sharif Naeini et al., 2006). These investigators demonstrated that the basis of the calcium influx may be an N-terminal splice variant of the TRPV1 channel. The variant channel is activated by cell shrinkage leading to an influx of calcium. Such a channel could underlie the effects of hyper-osmolar solutions on calcium influx and the BBB integrity of the NVU. However, it is currently not know if this splice variant of TRPV1 is expressed in the brain microvessel endothelial cells or whether splice variants of other TRPV channels may play a role in shrinkage-induced activation of calcium influx.

15.5 Conclusions

The BBB/NVU is a critical structure for CNS microenvironmental regulation and can respond to changes in mechanical stress in a number of ways. While the exact mechanism of transducing mechanical stress is not currently known, calcium-permeable channels of the TRP superfamily are attractive candidates for mediating some of this signaling. Endothelial cells of the NVU express several mechanosensitive TRP channels, including TRPC1, TRPV2 and TRPV4 (Fig. 15.3); the presence of other mechanosensitive TRP channels, such as TRPA1 or TRPP1, is currently unknown. Activation of mechanosensitive channels leads to calcium influx, triggering alterations in tight junction function and changes in BBB permeability. These TRP channels are positioned to detect changes in mechanical stress in the cerebral vasculature due to changes in shear stress/blood flow or blood pressure, as in stroke or hypertension, or changes in plasma osmolarity that can lead to alterations in endothelial cell volume. The precise regulatory mechanisms remain to be elucidated in future studies.

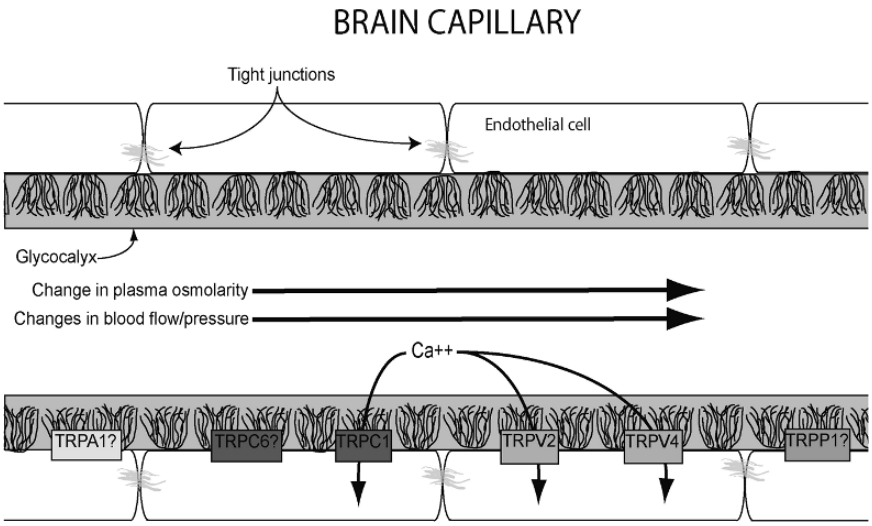

Fig. 15.3 Calcium influx trigger by mechanical stress at the blood-brain barrier. Changes in either blood flow or pressure (shear stress) or plasma osmolarity (osmolar stress) can trigger calcium influx in NVU endothelial cells through a number of the TRP family of ion channels. This calcium influx may be critical in the regulation of BBB functional integrity after alterations in mechanical stress in brain capillaries, such as loss of flow following stroke or hemorrhage

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