

The Blood-Brain Barrier/Neurovascular Unit in Health and Disease

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Abstract—The blood-brain barrier (BBB) is the regulated interface between the peripheral circulation and the central nervous system (CNS). Although originally observed by Paul Ehrlich in 1885, the nature of the BBB was debated well into the 20th century. The anatomical substrate of the BBB is the cerebral microvascular endothelium, which, together with astrocytes, pericytes, neurons, and the extracellular matrix, constitute a “neurovascular unit” that is essential for the health and function of the CNS. Tight junctions (TJ) between endothelial cells of the BBB restrict paracellular diffusion of water-soluble substances from blood to brain. The TJ is

an intricate complex of transmembrane (junctional adhesion molecule-1, occludin, and claudins) and cytoplasmic (zonula occludens-1 and -2, cingulin, AF-6, and 7H6) proteins linked to the actin cytoskeleton. The expression and subcellular localization of TJ proteins are modulated by several intrinsic signaling pathways, including those involving calcium, phosphorylation, and G-proteins. Disruption of BBB TJ by disease or drugs can lead to impaired BBB function and thus compromise the CNS. Therefore, understanding how BBB TJ might be affected by various factors holds significant promise for the prevention and treatment of neurological diseases.

I. The Concept of the Blood-Brain Barrier

A. Initial Observations of the Blood-Brain Barrier

The central nervous system (CNS¹) is the most critical and sensitive system in the human body. Proper neuronal function necessitates a highly regulated extracellular environment, wherein the concentrations of ions such as Na⁺, K⁺, and Ca²⁺ must be maintained within very narrow ranges. Furthermore, the metabolic demands of nervous tissue are considerable, with the CNS accounting for approximately 20% of oxygen consumption in humans (Rolfe and Brown, 1997). The CNS is also extremely sensitive to a wide range of chemicals; many of the substances we consume in our diet, although readily metabolized and excreted without harm to peripheral organ systems, are in fact quite neurotoxic.

It is therefore essential that the interface between the CNS and the peripheral circulatory system functions as a dynamic regulator of ion balance, a facilitator of nutrient transport, and a barrier to potentially harmful molecules. This homeostatic aspect of the cerebral microcirculation, historically referred to as the "blood-brain barrier" (BBB), performs all of these functions.

The first experimental evidence of the BBB was described by Paul Ehrlich, who noted that water-soluble dyes injected into the circulatory system stained all organs except the brain and spinal cord (Ehrlich, 1885). Ehrlich attributed this observation to a low affinity of nervous tissue to the dye (Ehrlich, 1904). Subsequent experiments by Ehrlich's student Edwin Goldmann showed that this was not the case; injection of trypan blue directly into the cerebrospinal fluid (CSF) stained all cell types in the brain but failed to penetrate into the periphery (Goldmann, 1913). This suggested that there existed a barrier to the dye between the CNS and the peripheral circulation. Lewandowsky was the first to use the term *bluthirnschranke* (blood-brain barrier) while studying the limited permeation of potassium ferrocyanate into the brain (Lewandowsky, 1900).

B. Experimental Controversies

Although Goldmann's work strongly suggested the existence of a physical barrier between the CNS and the circulation, the nature of this barrier—and indeed its existence—were debated well into the 1960s. One criticism of Goldmann's experiment was that the compositions of blood and CSF were sufficiently different as to influence the diffusibility of dyes and/or their affinity for nervous tissue; thus, it was improper to compare injections into the bloodstream with injections into the CSF.

¹Abbreviations: CNS, central nervous system; BBB, blood-brain barrier; CSF, cerebrospinal fluid; EM, electron microscopy; TJ, tight junction(s); AJ, adherens junction(s); VE, vascular endothelial; JAM, junctional adhesion molecule; ZO, zonula occludens; MAGUK, membrane-associated guanylate kinase-like; PKC, protein kinase C; SKF 96365, 1-(β-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl)-1H-imidazole; MS, multiple sclerosis; HIV, human immunodeficiency virus.

The situation was further complicated by the observation that basic aniline dyes crossed the apparent BBB and stained the brain, although acidic aniline dyes did not. In a review of the known permeabilities of a variety of substances, Friedemann (1942) concluded that "the ability of substances to pass the capillaries of the C-N-S is determined by their electrochemical properties. The cerebral capillaries are permeable to substances carrying a positive or no charge at the pH of blood while they are impermeable to those carrying a negative charge. . . the problem is only part of the more general problem of capillary permeability. . . ."

This hypothesis proved inadequate as the rates of exchange of more solutes between the blood and CNS were measured; subsequent attempts to describe the physiochemical properties of molecules that determined their rate of entry into the brain included hypotheses based on molecular weight, molecular size, binding affinities, dissociation constants, lipid solubility, electric charge, and various combinations of all of these (Tschirgi, 1962).

In addition to the perplexing apparent selectivity of the BBB, there was much controversy over the specific anatomical structure(s) that determined the rates of solute exchange. The advent of electron microscopy (EM) enabled more detailed anatomical studies of the brain and its vasculature. An early EM study by Maynard et al. (1957) seemed to indicate that the interstitial space of the brain was virtually nonexistent and, therefore, the BBB was essentially an artifact:

"The present authors believe that the 'blood-brain barrier' may be mainly an illusion. Physiologists have been led to postulate its existence on the assumption that there are considerable extracellular tissue spaces. Which would be in equilibrium with blood plasma if a barrier did not exist. We see, however, that the basic assumption is incorrect and that these tissue spaces simply do not exist."

"When the physiologist quantitatively studies the penetration of ions, organic crystalloids, proteins, vital dyes, etc. into nervous tissue, he must actually be studying the penetration (or lack of it) into the glial nervous cells. . . Thus, we believe that the physiological data available should be re-interpreted on the assumption that nearly all substances are within cytoplasmic compartments. Under these circumstances there would be no expectation of a simple correlation with their distribution into the blood plasma. The commonly accepted laws of permeability, which apply elsewhere in the body, may thus indeed apply in the brain without important exception and without invoking a specialized barrier."

Although a number of investigators supported the idea that the apparent barrier was merely a function of limited or nonexistent extracellular space in the brain, Dobbing (1961) pointed out that this made no sense in light of the findings of Hodgkin (1951) and others regarding the electrolyte gradients necessary for neural

function to occur. Hugh Davson argued (Davson and Spaziani, 1959) that if the slow passage of certain materials from the circulation into the brain were simply due to the necessity of penetrating parenchymal cells rather than a barrier at the level of the capillary endothelium or its basement membrane, then these materials would equilibrate at a similarly slow rate from a Ringer solution into excised brain tissue where the vasculature was no longer interposed. Furthermore, these materials would equilibrate quickly into excised skeletal muscle under the same conditions due to the presence of an ample extracellular space. In testing this hypothesis with iodide, *p*-aminohippurate, and sucrose, it was found that this was not the case. Penetration into the brain following peripheral injection occurred at “barely measurable rates by contrast with their penetration into skeletal muscle” (Davson and Spaziani, 1959). However, when excised cerebral hemispheres were incubated in Ringer solution containing these substances, equilibration occurred at a rate and magnitude comparable to that of skeletal muscle, indicating an extracellular space in the brain similar to that in muscle (14–22%) that is blocked *in vivo* by a barrier to diffusion from the vasculature (Davson and Spaziani, 1959). Further support for this idea was obtained in an elegant study by Hoffman and Olszewski (1961), in which it was shown that sodium fluorescein diffused from the area postrema—a region of the brain that lacks BBB properties—into surrounding tissues. Since fluorescein was not confined to the “barrier-less” region of the brain, it was concluded that the barrier must be due to special properties of the vascular system rather than a lack of extracellular space in the brain.

Another controversy was whether the anatomical structure forming the BBB was the capillary endothelium itself, the astrocytic processes that ensheath the capillaries, or the basal lamina (with which the astrocyte end-feet are continuous). The close apposition of astrocytic processes to capillaries as well as the large percentage of the capillary surface covered by these processes certainly seemed to indicate that astrocytes were involved in the anatomical barrier, if not its sole constituent. Indeed, a number of experimental findings supported this idea as well (reviewed in de Robertis and Gerschenfeld, 1961). However, EM studies by Reese and Karnovsky (1967) at a resolution of 100,000 \times that could distinguish between the capillary lumen and the space between the end-feet/basal lamina and outer wall of the capillary demonstrated that horseradish peroxidase administered via the vasculature did not pass the lumen. Furthermore, the presence of epithelial-like “tight junctions” (TJ) was reported in the interendothelial cleft. Thus, it was proposed that the capillary lumen bridged by TJ formed a continuous, impermeable membrane that constituted the primary anatomical substrate of the BBB (Reese and Karnovsky, 1967). Later studies showed that horseradish peroxidase injected into the

brain could diffuse through the \sim 20-nm gaps between astrocyte end-feet to the abluminal surface of the endothelium, indicating that astrocytes do not significantly contribute to the physical barrier (Brightman and Reese, 1969).

C. Anatomical Description

Although the concept of the BBB has continued to be refined over the past few decades, the current understanding of its basic structure is built on the general framework established by the work of Reese, Karnovsky, and Brightman in the late 1960s. Specifically, the BBB exists primarily as a selective diffusion barrier at the level of the cerebral microvascular endothelium, characterized by the presence of tight cell-cell junctions and a lack of fenestrations. Figure 1 shows a schematic cross-sectional representation of a typical cerebral capillary. The circumference of the capillary lumen is enclosed by a single endothelial cell. Anatomically, the endothelial cells of the BBB are distinguished from those in the periphery by increased mitochondrial content (Oldendorf et al., 1977), a lack of fenestrations (Fenstermacher et al., 1988), minimal pinocytotic activity (Sedlakova et al., 1999), and the presence of TJ (Kniesel and Wolburg, 2000). Attached at irregular intervals to the abluminal surface of the endothelium are pericytes, often divided into granular and filamentous subtypes (Tagami et al., 1990). Pericytes and endothelial cells are ensheathed by the basal lamina, a membrane 30 to 40-nm thick composed of collagen type IV, heparin sulfate proteoglycans, laminin, fibronectin, and other extracellular matrix proteins (Farkas and Luiten, 2001). The basal

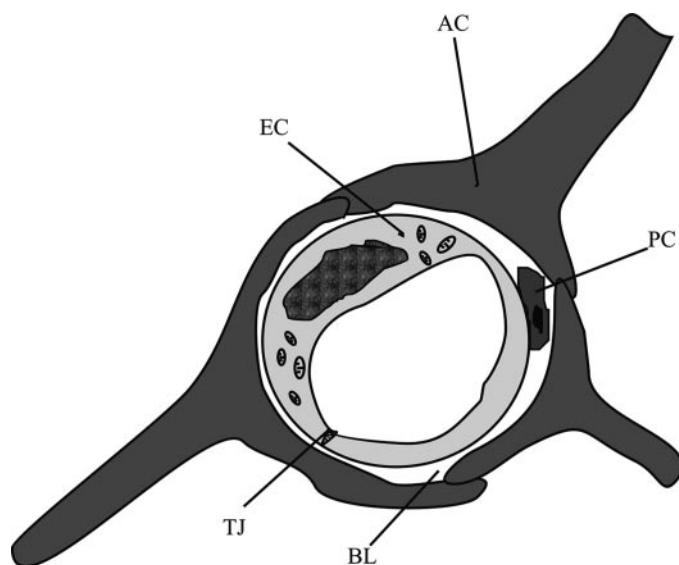


FIG. 1. Schematic cross-sectional representation of a cerebral capillary. The circumference of the capillary lumen is completely surrounded by a single endothelial cell (EC), the apposing membranes of which are connected by TJ. Pericytes (PC) are attached to the abluminal surface of the EC, and these two cell types are surrounded by the basal lamina (BL), which is contiguous with the plasma membranes of astrocyte (AC) end-feet and ECs.

lamina is contiguous with the plasma membranes of astrocyte end-feet, which ensheath the cerebral capillaries.

II. The Neurovascular Unit: Interactions of the Microvascular Endothelium with Neural Tissue

A. Astrocytes

The unique morphology and functionality of the cerebral microvascular endothelium suggests that cerebral endothelial cells are intrinsically unique or that the cellular milieu of the brain somehow induces BBB characteristics. Given the close anatomical association of astrocytes described in the previous section, it has long been thought that astrocytes are critical in the development and/or maintenance of BBB characteristics (Davson and Oldendorf, 1967). A number of ectopic grafting studies and *in vitro* experiments have supported this idea; however, recent discoveries have necessitated a broader view of the role of astrocytes at the BBB, as well as a reexamination of the roles of other parenchymal cells and the extracellular matrix.

In a critical experiment by Stewart and Wiley (1981), grafts of immature, nonvascularized brain tissue were transplanted from embryonic quails into the coelomic cavity of chick embryos; similarly, somite (dorsal mesoderm) grafts were transplanted from embryonic quails to the cerebral ventricles of chick embryos. The fact that the grafts were not yet vascularized coupled with the unique morphology of quail cell nuclei (Le Douarin, 1973) enabled the investigators to definitively determine the origin of vascular tissues invading the grafts. Both types of grafts were successful, and both were well vascularized by the surrounding host tissue. It was found that brain tissue grafted into the coelomic cavity developed capillaries with "thick basement membranes, and endothelial cells with high mitochondrial densities but relatively few pinocytotic vesicles and linked by tight junctions," whereas the capillaries in the grafted somites "were found to have a low mitochondrial density but relatively large numbers of pinocytotic vesicles. . . tight junctions were. . . not a common occurrence" (Stewart and Wiley, 1981). Furthermore, trypan blue injected into the graft sites indicated that vessels invading brain grafts developed BBB-like restricted permeability, whereas brain vessels invading somite grafts were quite leaky. Thus, it was concluded that the interaction of developing vascular tissue with nervous tissue is critical in the development of the BBB.

The potential role of astrocytes in this process was demonstrated by the injection of purified neonatal astrocytes into the anterior chamber of the eye. Injected astrocytes formed aggregates that were quickly vascularized; intravenous injection of Evans blue showed that the new vessels excluded the dye from the surrounding astrocytes, similar to what is observed in the brain (Janzer and Raff, 1987). Similarly, culture of brain endothelial cells with astrocytes (Tao-Cheng et al., 1987; Neuhaus et al., 1991) or with

astrocyte-conditioned media (Maxwell et al., 1987) has been shown to improve BBB characteristics *in vitro*. Nonetheless, experiments involving coculture of brain endothelial cells with immature astrocytes have been criticized as inconclusive on methodological grounds, specifically that astrocytes may merely help *maintain* BBB morphology in culture (Holash et al., 1993). Subsequent *in vivo* studies have indicated that cerebral microvessels can survive and maintain barrier integrity in areas of the brain that have experienced extensive astrocyte loss (Krum et al., 1997), although these results are in conflict with a more recent study that showed loss and restoration of barrier integrity *in vivo* following a temporary focal loss of astrocytes (Willis et al., 2004). It has also been speculated that astrocytes may act as intermediaries to or in conjunction with neurons in the moment-to-moment regulation of cerebral microvascular permeability (Ballabh et al., 2004), in particular via dynamic Ca^{2+} signaling between astrocytes and the endothelium via gap junctions and purinergic transmission (Braet et al., 2001; Zonta et al., 2003).

B. Pericytes

Less is known about the involvement of pericytes at the BBB. Contractile proteins have been found in cerebral pericytes, indicating that they may regulate capillary blood flow (Bandopadhyay et al., 2001). The addition of pericytes to cocultures of endothelial cells and astrocytes seems to stabilize the formation of capillary-like structures (Ramsauer et al., 2002). Pericytes have also been shown to migrate away from brain microvessels in rapid response to hypoxia (Gonul et al., 2002) and traumatic brain injury (Dore-Duffy et al., 2000); both of these conditions are associated with increased BBB permeability. Whether pericyte migration plays a causative role in BBB failure has not yet been established. However, pericyte-derived angiopoietin can induce endothelial expression of occludin, a major constituent of BBB TJ (Hori et al., 2004), indicating that pericytes are involved in the induction and/or maintenance of barrier properties in the cerebral endothelium in a manner similar to glia.

C. Neurons

Given the dynamic nature of neural activity and the considerable metabolic needs of nervous tissue, the microcirculation of the brain must be highly responsive to the tissue it supplies. Indeed, "metabolic coupling" of regional brain activity to blood flow is the basis of functional neuroimaging (Buxton and Frank, 1997), although the cellular mechanisms of this process are not well established (Pae-meleire, 2002). Interestingly, disruption of BBB integrity often accompanies pathological changes in cerebral blood flow and perfusion pressure (i.e., ischemia, hemorrhage, or traumatic injury) (Hatashita and Hoff, 1990; Petty and Wettstein, 2001), and there is evidence that such BBB opening may be a selective, compensatory event rather than a simple anatomical disruption (Lee et al., 1999). This implies that communication between neurons and the vas-

culature may not simply regulate blood flow, but BBB permeability as well. Anatomical evidence has been found for direct innervation of the microvascular endothelium and/or associated astrocytic processes by noradrenergic (Ben-Menachem et al., 1982; Cohen et al., 1997), serotonergic (Cohen et al., 1996), cholinergic (Vaucher and Hamel, 1995; Tong and Hamel, 1999), and GABA-ergic (Vaucher et al., 2000) neurons, as well as others (Kobayashi et al., 1985). Chemical lesion of the locus coeruleus—from which the noradrenergic projections to the vasculature originate—increases the vulnerability of the BBB to acute hypertension (Ben-Menachem et al., 1982). Also, significant loss of cholinergic innervation of cortical microvessels has been observed in Alzheimer's disease, which may lead to impaired cerebrovascular function observed in this disease (Tong and Hamel, 1999). Whether neurons are critical in the development of the BBB phenotype has not yet been demonstrated, but it is apparent that they can regulate critical aspects of BBB function.

D. The Extracellular Matrix

In addition to astrocytes, pericytes, and neurons, the extracellular matrix of the basal lamina also interacts with the cerebral microvascular endothelium. Disruption of the extracellular matrix is strongly associated with increased BBB permeability in pathological states (Rosenberg et al., 1993; Rascher et al., 2002). The extracellular matrix seems to serve as an anchor for the endothelium via interaction of laminin and other matrix proteins with endothelial integrin receptors (Hynes, 1992). Such cell-matrix interactions can stimulate a number of intracellular signaling pathways (reviewed in Tilling et al., 2002). Matrix proteins can influence the

expression of endothelial TJ proteins (Tilling et al., 1998; Savettieri et al., 2000), indicating that although the TJ constitute the primary impediment to paracellular diffusion, the proteins of the basal lamina are likely involved in their maintenance.

In light of these discoveries, it has been proposed that the microvascular endothelium, astrocytes, pericytes, neurons, and extracellular matrix constitute a “neurovascular unit” and that this view of the BBB is critical to understanding its development and physiology (Cohen et al., 1996; Neuwelt, 2004; Wang et al., 2004). Furthermore, the concept of the neurovascular unit establishes a framework for an integrative approach to understanding how the brain responds to cerebrovascular pathology (Lo et al., 2004). Finally, the neurovascular unit concept provides a basis for understanding the multiple pathways by which cerebral microvascular permeability could be regulated by drugs or disease (Fig. 2).

III. Molecular Physiology and Pathophysiology of the Blood-Brain Barrier Junctional Complex

A. Junctions of the Blood-Brain Barrier

The interendothelial space of the cerebral microvasculature is characterized by the presence of a junctional complex that includes adherens junctions (AJ) (Schulze and Firth, 1993), TJ (Kniesel and Wolburg, 2000; Wolburg and Lippoldt, 2002; Vorbrott and Dobrogowska, 2003), and possibly gap junctions (Tao-Cheng et al., 1987; Braet et al., 2001; Kojima et al., 2003; Simard et al., 2003). Whereas gap junctions (if they are present at interendothelial junctions of the BBB) mediate intercellular communication, both AJ and TJ act to restrict

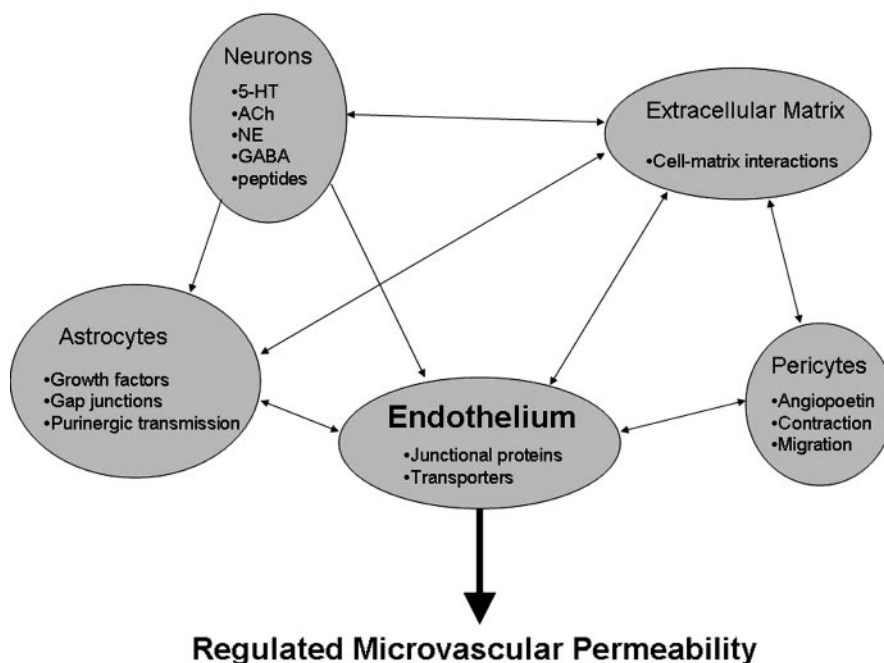


FIG. 2. The neurovascular unit. Shown are examples of how each of the various components of the neurovascular unit might contribute to the dynamic regulation of microvascular permeability.

permeability across the endothelium (Bazzoni and Dejana, 2004).

AJ are ubiquitous in the vasculature and mediate the adhesion of endothelial cells to each other, contact inhibition during vascular growth and remodeling, initiation of cell polarity, and (in part) the regulation of paracellular permeability (Brown and Davis, 2002; Bazzoni and Dejana, 2004). The primary component of AJ is vascular endothelial (VE)-cadherin, a Ca^{2+} -regulated protein that mediates cell-cell adhesion via homophilic interactions between the extracellular domains of proteins expressed in adjacent cells (Vincent et al., 2004). The cytoplasmic tail of VE-cadherin binds to β -catenin and plakoglobin, which in turn bind via α -catenin, α -actinin, and vinculin to the actin cytoskeleton, stabilizing the AJ complex (Knudsen et al., 1995; Lampugnani et al., 1995; Watabe-Uchida et al., 1998).

Although disruption of AJ at the BBB can lead to increased permeability (Abbruscato and Davis, 1999b), it is primarily the TJ that confers the low paracellular permeability and high electrical resistance (Romero et al., 2003). TJ are elaborate structures that span the apical region of the intercellular cleft of epithelial and endothelial barrier tissues (Fig. 3). They function both as a “zipper” that affects separation of the apical and basolateral cell membranes, enabling asymmetric distri-

bution of membrane constituents, and a “fence” that limits paracellular permeability. Much like AJ, TJ are composed of transmembrane proteins that form the primary seal linked via accessory proteins to the actin cytoskeleton (Vorbrot and Dobrogowska, 2003).

B. Proteins of the Tight Junction

1. *Junctional Adhesion Molecules.* The transmembrane components of the TJ at the BBB include junctional adhesion molecule (JAM)-1 (Del Maschio et al., 1999), occludin, and the claudins. JAM-1 is a 40-kDa member of the IgG superfamily and is believed to mediate the early attachment of adjacent cell membranes via homophilic interactions (Dejana et al., 2000). JAM-1 is composed of a single membrane-spanning chain with a large extracellular domain (Martin-Padura et al., 1998). Related proteins JAM-2 and JAM-3 are also present in endothelial tissues and lymphatic cells, but not epithelia (Bauer et al., 2004). In addition to their developmental roles, JAMs may regulate the transendothelial migration of leukocytes (Del Maschio et al., 1999), but their function in the mature BBB is still largely unknown.

2. *Occludin.* Occludin, a 60- to 65-kDa protein, has four transmembrane domains with the carboxyl and amino terminals oriented to the cytoplasm and two extracellular loops that span the intercellular cleft (Furuse

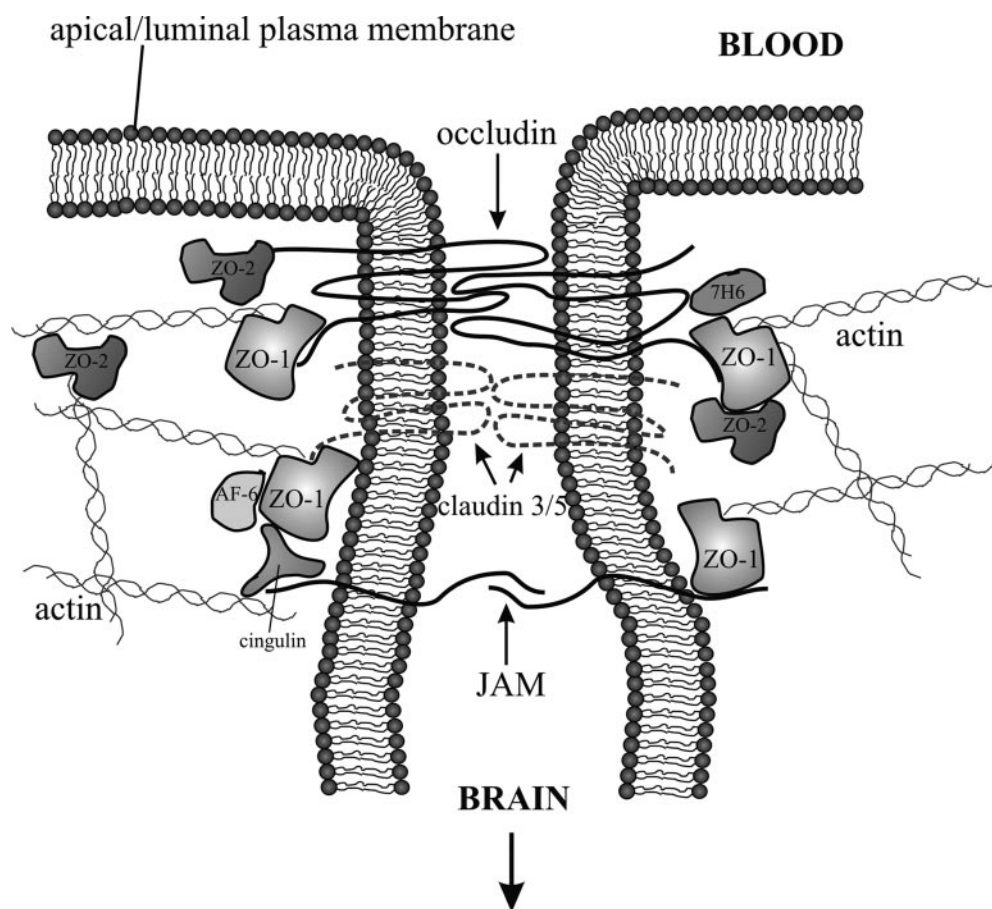


FIG. 3. Basic molecular organization of blood-brain barrier tight junctions.

et al., 1993). It is highly expressed and consistently stains in a distinct, continuous pattern along the cell margins in the cerebral endothelium (Lippoldt et al., 2000; Hawkins et al., 2004), whereas it is much more sparsely distributed in non-neural endothelia (Hirase et al., 1997). Occludin increases electrical resistance in TJ-containing tissues (McCarthy et al., 1996), a property mediated by the second extracellular loop domain (Wong and Gumbiner, 1997). Occludin has multiple sites for phosphorylation on serine and threonine residues; the phosphorylation state of occludin seems to be involved in the regulation of its association with the cell membrane, although the precise mechanisms vary among tissues and species (Sakakibara et al., 1997; Wachtel et al., 1999; Clarke et al., 2000; Andreeva et al., 2001; Hirase et al., 2001; Rao et al., 2002; Kale et al., 2003). The cytoplasmic C-terminal domain is likely involved in the association of occludin with the cytoskeleton via accessory proteins [such as zonula occludens (ZO)-1 and ZO-2] (Furuse et al., 1994; Fanning et al., 1998). Expression of C-terminal truncated versions of occludin leads to increased paracellular permeability to low molecular weight molecules (Balda et al., 1996). Interestingly, results of several knockout and knockdown experiments indicate that occludin is not essential for the formation of TJ, despite the fact that decreased expression of occludin is associated with disrupted BBB function in a number of disease states (Bolton et al., 1998; Huber et al., 2002; Brown and Davis, 2005).

3. Claudins. Claudins have a similar membrane topography to occludin but no sequence homology (Furuse et al., 1998). Claudins are 20- to 24-kDa proteins, of which at least 24 have been identified in mammals (Bauer et al., 2004). All claudins have similar predicted folding and high sequence homology among themselves in the first and fourth transmembrane domains and extracellular loops (Heiskala et al., 2001). The extracellular loops of claudins interact via homophilic and heterophilic interactions between cells (Furuse et al., 1999). Overexpression of claudin species in fibroblasts can induce cell aggregation and formation of TJ-like strands. Conversely, expression of occludin does not lead to the formation of TJ; rather, occludin only localizes to TJ in cells that have already been transfected with claudins (Kubota et al., 1999). Thus, it is hypothesized that claudins form the primary “seal” of the TJ, and occludin acts as an additional support structure. The expression patterns of claudins vary among different tissues; in the cerebral microvascular endothelium, claudin-1, -3, and -5 have been detected, although the expression of claudin-1 at the BBB has been called into question (Morcos et al., 2001; Nitta et al., 2003; Witt et al., 2003; Wolburg et al., 2003; Hawkins et al., 2004).

4. Membrane-Associated Guanylate Kinase-Like Proteins. In addition to the transmembrane components of the TJ, there are several accessory proteins that associate with them in the cytoplasm. These include members

of the membrane-associated guanylate kinase-like (MAGUK) homolog family. MAGUK proteins are characterized by multiple postsynaptic density protein-95/discs-large/ZO-1 binding domains, an Src homolog-3 domain, and a guanylate kinase-like domain. These domains enable multiple protein-protein interactions (Shin et al., 2000). MAGUK proteins seem to be involved in the coordination and clustering of protein complexes to the cell membrane and in the establishment of specialized domains within the membrane (Gonzalez-Mariscal et al., 2000). Three MAGUK proteins have been identified at the TJ: ZO-1, ZO-2, and ZO-3.

ZO-1 was the first protein positively associated with the TJ (Stevenson et al., 1986). It is a 220-kDa phosphoprotein expressed in endothelial and epithelial cells as well as in many cell types that do not have TJ (Howarth et al., 1992). In addition to its association with TJ, it has been found to be associated with AJ (Itoh et al., 1993) and gap junction (Toyofuku et al., 1998) proteins. ZO-1 links transmembrane proteins of the TJ to the actin cytoskeleton (Fanning et al., 1998). This interaction is likely critical to the stability and function of the TJ, because dissociation of ZO-1 from the junctional complex is often associated with increased permeability (Abbruscato et al., 2002; Fischer et al., 2002; Mark and Davis, 2002). ZO-1 may also act as a signaling molecule that communicates the state of the TJ to the interior of the cell, or vice versa. ZO-1 has been shown to localize to the nucleus under conditions of proliferation and injury (Gottardi et al., 1996), following Ca^{2+} depletion (Riesen et al., 2002), and in response to nicotine (Hawkins et al., 2004). It has also been colocalized with transcription factors (Balda and Matter, 2000) and G-proteins (Meyer et al., 2002).

ZO-2, a 160-kDa phosphoprotein with a high-sequence homology to ZO-1, coprecipitates with ZO-1 (Gumbiner et al., 1991). Less is known about the function of ZO-2, although like ZO-1, it binds to both structural constituents of the TJ and signaling molecules such as transcription factors (Betanzos et al., 2004) and localizes into the nucleus during stress and proliferation (Islas et al., 2002; Traweger et al., 2002). In cultured brain microvessel endothelial cells, ZO-2 is localized along the membrane at cell-cell contacts (Mark and Davis, 2002), although in cerebral microvessel fragments it seems to be much more diffusely distributed (Hawkins et al., 2004). Interestingly, ZO-2 may function somewhat redundantly with ZO-1, replacing it and facilitating formation of morphologically normal TJ in cultured epithelia lacking ZO-1 (Umeda et al., 2004). ZO-2 has also been found in non-TJ-containing tissues (Itoh et al., 1999). ZO-3 is a 130-kDa homolog that has been found in some TJ-containing tissues but, to date, not at the BBB (Inoko et al., 2003).

5. Other Accessory Proteins. Other accessory proteins of the BBB include cingulin, AF-6, and 7H6. Cingulin is a 140- to 160-kDa protein that associates with

ZOs, JAM-1, and myosin (Citi et al., 1989) and is hypothesized to mediate interactions between the cytoskeleton and the TJ via force transduction (Bauer et al., 2004). AF-6, a 180-kDa protein with two Ras-associating domains, interacts with ZO-1. This interaction is inhibited by Ras activation, indicating that disruption of the ZO-1/AF-6 complex may be critical in the modulation of TJ via pathways that involve Ras (Yamamoto et al., 1999). The function of 7H6 is not known, but this 155-kDa protein reversibly dissociates from the TJ complex under conditions of ATP depletion (Zhong et al., 1994). In addition to these, some recently identified proteins associated with the TJ (none characterized at the BBB to date) are listed in Table 1.

C. Intracellular Signaling Pathways Involved in Tight Junction Organization and Function

TJ are dynamic structures. Proteins of the TJ are subject to changes in expression, subcellular localization, post-translational modification, and protein-protein interactions under both physiological and pathophysiological conditions (Huber et al., 2001a). The intracellular signaling pathways that regulate these processes are only beginning to be elucidated, with most of the work being done in epithelial tissues. At the BBB, it is becoming clear that responses of TJ to various stimuli can in fact be mediated by multiple pathways simultaneously (Fischer et al., 2004). The interactions of TJ proteins with each other and with intracellular signaling pathways have been reviewed extensively elsewhere (Bazzoni and Dejana, 2004); as such, we limit our treatment here to pathways demonstrated to be involved in the regulation of TJ organization and function at the BBB specifically.

1. Calcium. Even before specific proteins of the TJ were well characterized, it was known that both extracellular Ca^{2+} depletion and increased intracellular $[\text{Ca}^{2+}]$ could lead to disruption of BBB TJ (Nagy et al., 1985; Abbott and Revest, 1991). Epithelia cultured in Ca^{2+} -deficient media demonstrate a loss of ZO-1, ZO-2, and occludin from the cell membrane and an associated increase in paracellular permeability; these effects are attenuated by protein kinase A inhibitors (Klingler et al., 2000). Protein kinase C (PKC) activation can also overcome the deleterious effect of low extracellular $[\text{Ca}^{2+}]$, indicating that PKC is involved in Ca^{2+} -dependent regulation of TJ (Balda et al., 1993). Interestingly,

both abnormally high (Stuart et al., 1996) and abnormally low (Ye et al., 1999) intracellular $[\text{Ca}^{2+}]$ can disrupt the TJ via decreased expression and/or disrupted protein-protein interactions, indicating a role for intracellular stores in the regulation of TJ. In addition to PKC, Ca^{2+} influx or release from intracellular stores can activate any number of kinase signaling cascades, leading to the activation of transcription factors (nuclear factor- κB , cAMP response element-binding protein, c-fos, etc.) that regulate TJ protein expression (Brown and Davis, 2002). Interestingly, although artificially raising intracellular $[\text{Ca}^{2+}]$ does not alter BBB TJ (Brown et al., 2004), treatment with the Ca^{2+} channel blocker SKF 96365 ameliorates the permeability-increasing effects of hypoxia/aglycemia on the BBB via a mechanism that seems to involve recruitment of occludin to the cell membrane (Brown and Davis, 2005).

2. Phosphorylation. Phosphorylation is a major regulatory mechanism of both transmembrane and accessory proteins at the TJ (Staddon et al., 1995; Sakakibara et al., 1997). For example, serine phosphorylation regulates the subcellular localization of occludin (Andreeva et al., 2001), and both serine and threonine phosphorylation of occludin are highly correlated with the TJ reassembly following disruption (Farshori and Kachar, 1999; Tsukamoto and Nigam, 1999). cAMP treatment of cultured brain endothelial cells leads to enhanced immunoreactivity of claudin-5 at the junctional complex via a protein kinase A-dependent mechanism (Ishizaki et al., 2003). Early evidence suggested that junctional permeability was correlated with the phosphate content of ZO-1 (Stevenson et al., 1989), although subsequent studies have indicated that phosphorylation of ZO-1 is critical to its membrane targeting during the development of TJ (Kurihara et al., 1995). Given the multiple sites for phosphorylation on TJ proteins, and in particular on occludin and ZO-1 (Sakakibara et al., 1997), it is highly unlikely that any clear, one-to-one correspondence exists between phosphorylation and TJ formation and function. More likely, phosphorylation of distinct serine, tyrosine, and threonine residues has distinct structural and functional effects.

3. G-Proteins. G-proteins are also involved in the development and modulation of TJ (Balda et al., 1991; Saha et al., 1998; Hopkins et al., 2000). Heterotrimeric G-proteins are expressed by cerebral endothelial cells (Fabian et al., 1998), where they mediate migration of

TABLE 1
Recently identified TJ-associated proteins

Protein	Tissue	Homology	Function	Reference
LYRIC	Epithelial	None	Unknown; associates with occludin and ZO-1	Britt et al. (2004)
JACOP	Epithelial and endothelial	Cingulin	Possible link between TJ and cytoskeleton	Ohnishi et al. (2004)
Moe	Retinal epithelium	?	Necessary for TJ formation in the retina	Jensen and Westerfield (2004)
CLMP	Epithelial cell lines	CTX family	Cell adhesion; increases electrical resistance	Raschperger et al. (2004)
JAM-4	Kidney	IgG superfamily	Cell adhesion; regulates permeability	Hirabayashi et al. (2003)
JEAP	Exocrine cells	?	Unknown; associates with TJ in exocrine cells	Nishimura et al. (2002)
R4.1	MDCK cells	?	Possible link between TJ and cytoskeleton	Mattagajasingh et al. (2000)

T-lymphocytes into the brain (Adamson et al., 2002). The Rho family of GTPases has been implicated in the alteration of TJ permeability via interactions with the actin cytoskeleton, acting downstream of G-protein-coupled receptors for lysophosphatidic acid (Schulze et al., 1997) and prostaglandin E₂ (Hasegawa et al., 1999). Rab proteins, primarily known as regulators of vesicle trafficking, have been implicated in targeting TJ proteins such as ZO-1 to the junctional complex (Stevenson and Keon, 1998). The TJ accessory protein AF-6 interacts with ZO-1 and is a target for Ras (Yamamoto et al., 1999). Activation of Ras is associated with disruption of cell-cell contacts, loss of ZO-1 from the junctional complex, and increased permeability.

D. Alteration of Blood-Brain Barrier Tight Junction in Disease

Failure of the BBB is a critical event in the development and progression of several diseases that affect the CNS. In some cases, increased BBB permeability is a consequence of the pathology, such as with ischemic stroke (Ilzecka, 1996) and traumatic brain injury (Morganti-Kossmann et al., 2002), whereas in other cases BBB opening may be a precipitating event, such as with multiple sclerosis (MS) (de Vries and Dijkstra, 2004). In other conditions in which cerebrovascular abnormalities have been noted, such as Alzheimer's disease, the relationship between BBB breakdown and pathology is not clear (Wardlaw et al., 2003). Moreover, the contribution of specific BBB TJ alterations to neurological conditions is a rapidly developing area of inquiry.

1. Hypoxia/Ischemia. Cerebral ischemia is a complex insult that involves a loss of blood flow as well as depletion of oxygen and essential nutrients (del Zoppo and Hallenbeck, 2000) and is associated with increased microvascular permeability (Kempinski, 2001; Petty and Wettstein, 2001). A number of studies using in vitro models of the BBB have indicated that hypoxia and hypoxia/reoxygenation lead to increased permeability and/or disruption of BBB TJ (Abbruscato and Davis, 1999a; Fischer et al., 1999; Mark and Davis, 2002), although hypoxic stress may increase permeability via the transcellular route as well (Plateel et al., 1997; Cippolla et al., 2004). Some reports have indicated that BBB endothelial cells are less responsive to hypoxic stress when cocultured with astrocytes (Kondo et al., 1996; Fischer et al., 2000) or pericytes (Hayashi et al., 2004); nonetheless, in vivo hypoxia/reoxygenation treatment is associated with increased BBB permeability and decreased expression of occludin (Witt et al., 2003), as well as activation of the transcription factors nuclear factor- κ B and hypoxia-inducible factor-1 (Witt et al., 2005). Hypoxic reorganization of BBB TJ seems to be mediated in part by vascular endothelial growth factor and nitric oxide (Fischer et al., 1999; Mark et al., 2004), and vascular endothelial growth factor antagonism reduces postischemic edema and injury in vivo (van Bruggen et

al., 1999), indicating that TJ disruption is likely involved in the progression of ischemic brain injury.

2. Inflammation. Inflammatory mediators are known modulators of BBB permeability (Abbott, 2000). As such, compromised BBB TJ are a hallmark of neuroinflammatory disease states (Petty and Lo, 2002). BBB disruption is well established as an early event in the progression of MS, with MRI studies indicating a compromised barrier preceding clinical symptoms (de Vries and Dijkstra, 2004). In experimental models of MS, BBB disruption is induced by T-cells (Seeldrayers et al., 1993) and monocytes (Morrissey et al., 1996). MS lesions are associated with loss of occludin and ZO-1 in the microvasculature (Bolton et al., 1998; McQuaid et al., 2002; Kirk et al., 2003) that is likely mediated by cytokines such as monocyte chemoattractant protein-1 (Song and Pachter, 2004), tumor necrosis factor- α , interleukin-1B, and interferon- γ (Minagar and Alexander, 2003). Similar observations have been made in postmortem examinations of brains from HIV encephalitis (Dallasta et al., 1999) and Alzheimer's disease (Fiala et al., 2002) patients.

Interestingly, inflammation occurring in the periphery also has profound effects on BBB TJ proteins. Huber et al. (2001b) showed that inflammatory pain induced by three mechanistically distinct agents (formalin, complete Freund's adjuvant, and λ -carrageenan) injected into the hind paw of a rat increased BBB permeability to sucrose. These permeability changes were associated with decreased expression of occludin and increased expression of ZO-1 (Huber et al., 2001b). A more detailed time-course study of the λ -carrageenan model revealed that BBB opening is biphasic, with the increase in ZO-1 corresponding only to the early phase and the loss of occludin corresponding to both phases (Huber et al., 2002). Additionally, immunoprecipitation indicated that ZO-1 became less associated with actin and more associated with ZO-2, suggesting a disruption of the interaction between the TJ complex and the cytoskeleton (Huber et al., 2002). It is unclear what the mechanism(s) for TJ alterations following peripheral inflammation might be; although the possibility exists that cytokines circulating from the site of inflammation to the brain may be involved, acute surgical pain stress has also been shown to increase BBB permeability (Oztas et al., 2004). Also, cortical spreading depression, a phenomenon associated with migraine, up-regulates matrix metalloproteases, which increase BBB permeability via proteolysis of the basal lamina and disruption of the TJ (Sanchez-del-Rio and Reuter, 2004), including a specific loss of ZO-1 in the area of affected cortex (Gursoy-Ozdemir et al., 2004). These studies suggest that BBB TJ might be subject to modulation via centrally mediated responses to nociceptive stimuli.

3. Other Insults. In addition to hypoxic/ischemic and inflammatory insults, the BBB can also be compromised by drugs of abuse. For example, cocaine can accelerate

the progression of HIV-associated dementia (Nath et al., 2001), a process that may involve both direct effects of cocaine on the endothelium as well as proinflammatory effects (Fiala et al., 1998; Zhang et al., 1998; Gan et al., 1999). Nicotine, a potentially vasoactive drug (Hawkins et al., 2002), alters the transport of glucose (Duelli et al., 1998a,b) and ions (Wang et al., 1994; Abbruscato et al., 2004) at the BBB. Nicotine also decreases the expression and marginal localization of ZO-1, which is associated with increased permeability in the in vitro BBB (Abbruscato et al., 2002). In the rat, nicotine treatment also alters cerebral microvascular distribution of ZO-1, diminishes immunoreactivity for claudin-3, and increases BBB permeability to sucrose (Hawkins et al., 2004).

Diabetes, a disease with well established microvascular effects and a known risk factor for ischemic stroke (Goldstein et al., 2001), is also associated with increased vascular permeability and reduced occludin content in the retinal microvasculature (which is similar to that of the brain) (Antonetti et al., 1998) and at the BBB (Chenhade et al., 2002). Many different types of brain tumors are characterized by edema, in which TJ abnormalities are thought to play at least a contributing role (Papadopoulos et al., 2004). Tumor microvascular occludin expression has been inversely correlated with contrast enhancement, indicating that the loss of occludin may underlie the development of edema (Papadopoulos et al., 2001), and a specific loss of claudin-3 has been observed in human glioblastoma multiforme (Wolburg et al., 2003). It has been suggested that the "incomplete" TJ characteristics of tumor vasculature are a result of the proangiogenic environment of tumor growth and/or the paucity of normal astrocytes (Papadopoulos et al., 2004).

IV. Conclusions

The functions of the BBB/neurovascular unit in neuropathology—both as a contributing factor to the development and progression of disease and in its responses to disease—indicate that the neurovascular unit is a therapeutic target unto itself (Neuwelt, 2004) and not merely an obstacle to CNS drug delivery. The numerous pathways by which specific TJ proteins are regulated and the specific effects of certain pathologies on TJ proteins suggest that therapies targeted to individual components of the TJ complex and its modulators hold significant promise for the treatment and prevention of neurological diseases.

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References

Abbott NJ (2000) Inflammatory mediators and modulation of blood-brain barrier permeability. *Cell Mol Neurobiol* **20**:131–147.
Abbott NJ and Revest PA (1991) Control of brain endothelial permeability. *Cerebrovasc Brain Metab Rev* **3**:39–72.

Abbruscato TJ and Davis TP (1999a) Combination of hypoxia/aglycemia compromises in vitro blood-brain barrier integrity. *J Pharmacol Exp Ther* **289**:668–675.
Abbruscato TJ and Davis TP (1999b) Protein expression of brain endothelial cell E-cadherin after hypoxia/aglycemia: influence of astrocyte contact. *Brain Res* **842**:277–286.
Abbruscato TJ, Lopez SP, Mark KS, Hawkins BT, and Davis TP (2002) Nicotine and cotinine modulate cerebral microvascular permeability and protein expression of ZO-1 through nicotinic acetylcholine receptors expressed on brain endothelial cells. *J Pharm Sci* **91**:2525–2538.
Abbruscato TJ, Lopez SP, Roder K, and Paulson JR (2004) Regulation of blood-brain barrier Na,K,2Cl-cotransporter through phosphorylation during in vitro stroke conditions and nicotine exposure. *J Pharmacol Exp Ther* **310**:459–468.
Adamson P, Wilbourn B, Etienne-Manneville S, Calder V, Beraud E, Milligan G, Couraud PO, and Greenwood J (2002) Lymphocyte trafficking through the blood-brain barrier is dependent on endothelial cell heterotrimeric G-protein signaling. *FASEB J* **16**:1185–1194.
Andreeva AY, Krause E, Muller EC, Blasig IE, and Utepergenov DI (2001) Protein kinase C regulates the phosphorylation and cellular localization of occludin. *J Biol Chem* **276**:38480–38486.
Antonetti DA, Barber AJ, Khin S, Lieth E, Tarbell JM, and Gardner TW (1998) Vascular permeability in experimental diabetes is associated with reduced endothelial occludin content: vascular endothelial growth factor decreases occludin in retinal endothelial cells. The Penn State Retina Research Group. *Diabetes* **47**:1953–1959.
Balda MS, Gonzalez-Mariscal L, Contreras RG, Macias-Silva M, Torres-Marquez ME, Garcia-Sainz JA, and Cerejido M (1991) Assembly and sealing of tight junctions: possible participation of G-proteins, phospholipase C, protein kinase C and calmodulin. *J Membr Biol* **122**:193–202.
Balda MS, Gonzalez-Mariscal L, Matter K, Cerejido M, and Anderson JM (1993) Assembly of the tight junction: the role of diacylglycerol. *J Cell Biol* **123**:293–302.
Balda MS and Matter K (2000) The tight junction protein ZO-1 and an interacting transcription factor regulate ErbB-2 expression. *EMBO (Eur Mol Biol Organ) J* **19**:2024–2033.
Balda MS, Whitney JA, Flores C, Gonzalez S, Cerejido M, and Matter K (1996) Functional dissociation of paracellular permeability and transepithelial electrical resistance and disruption of the apical-basolateral intramembrane diffusion barrier by expression of a mutant tight junction membrane protein. *J Cell Biol* **134**:1031–1049.
Ballabh P, Braun A, and Nedergaard M (2004) The blood-brain barrier: an overview: structure, regulation and clinical implications. *Neurobiol Dis* **16**:1–13.
Bandopadhyay R, Orte C, Lawrenson JG, Reid AR, De Silva S, and Allt G (2001) Contractile proteins in pericytes at the blood-brain and blood-retinal barriers. *J Neurocytol* **30**:35–44.
Bauer H-C, Traeweger A, and Bauer H (2004) Proteins of the tight junction in the blood-brain barrier, in *Blood-Spinal Cord and Brain Barriers in Health and Disease* (Sharma HS and Westman J eds) pp 1–10, Elsevier, San Diego.
Bazzoni G and Dejana E (2004) Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. *Physiol Rev* **84**:869–901.
Ben-Menachem E, Johansson BB, and Svensson TH (1982) Increased vulnerability of the blood-brain barrier to acute hypertension following depletion of brain noradrenaline. *J Neural Transm* **53**:159–167.
Betanzos A, Huerta M, Lopez-Bayghen E, Azuara E, Amerena J, and Gonzalez-Mariscal L (2004) The tight junction protein ZO-2 associates with Jun, Fos and C/EBP transcription factors in epithelial cells. *Exp Cell Res* **292**:51–66.
Bolton SJ, Anthony DC, and Perry VH (1998) Loss of the tight junction proteins occludin and zonula occludens-1 from cerebral vascular endothelium during neutrophil-induced blood-brain barrier breakdown in vivo. *Neuroscience* **86**:1245–1257.
Braet K, Paemeleire K, D'Herde K, Sanderson MJ, and Leybaert L (2001) Astrocyte-endothelial cell calcium signals conveyed by two signalling pathways. *Eur J Neurosci* **13**:79–91.
Brightman MW and Reese TS (1969) Junctions between intimately apposed cell membranes in the vertebrate brain. *J Cell Biol* **40**:648–677.
Britt DE, Yang DF, Yang DQ, Flanagan D, Callanan H, Lim YP, Lin SH, and Hixson DC (2004) Identification of a novel protein, LYRIC, localized to tight junctions of polarized epithelial cells. *Exp Cell Res* **300**:134–148.
Brown RC and Davis TP (2002) Calcium modulation of adherens and tight junction function: a potential mechanism for blood-brain barrier disruption after stroke. *Stroke* **33**:1706–1711.
Brown RC and Davis TP (2005) Hypoxia/aglycemia alters expression of occludin and actin in brain endothelial cells. *Biochem Biophys Res Commun* **327**:1114–1123.
Brown RC, Mark KS, Egleton RD, and Davis TP (2004) Protection against hypoxia-induced blood-brain barrier disruption: changes in intracellular calcium. *Am J Physiol Cell Physiol* **286**:C1045–C1052.
Buxton RB and Frank LR (1997) A model for the coupling between cerebral blood flow and oxygen metabolism during neural stimulation. *J Cereb Blood Flow Metab* **17**:64–72.
Chenhade JM, Haas MJ, and Mooradian AD (2002) Diabetes-related changes in rat cerebral occludin and zonula occludens-1 (ZO-1) expression. *Neurochem Res* **27**:249–252.
Cipolla MJ, Crete R, Vitullo L, and Rix RD (2004) Transcellular transport as a mechanism of blood-brain barrier disruption during stroke. *Front Biosci* **9**:777–785.
Citi S, Sabanay H, Kendrick-Jones J, and Geiger B (1989) Cingulin: characterization and localization. *J Cell Sci* **93**:107–122.
Clarke H, Soler AP, and Mullin JM (2000) Protein kinase C activation leads to dephosphorylation of occludin and tight junction permeability increase in LLC-PK1 epithelial cell sheets. *J Cell Sci* **113**:3187–3196.
Cohen Z, Bonvento G, Lacombe P, and Hamel E (1996) Serotonin in the regulation of brain microcirculation. *Prog Neurobiol* **50**:335–362.
Cohen Z, Molinatti G, and Hamel E (1997) Astroglial and vascular interactions of

- noradrenaline terminals in the rat cerebral cortex. *J Cereb Blood Flow Metab* **17**:894–904.
- Dallasta LM, Pisarova LA, Esplen JE, Werley JV, Moses AV, Nelson JA, and Achim CL (1999) Blood-brain barrier tight junction disruption in human immunodeficiency virus-1 encephalitis. *Am J Pathol* **155**:1915–1927.
- Davson H and Oldendorf WH (1967) Symposium on membrane transport. Transport in the central nervous system. *Proc R Soc Med* **60**:326–329.
- Davson H and Spaziani E (1959) The blood-brain barrier and the extracellular space of brain. *J Physiol* **149**:135–143.
- de Robertis E and Gerschenfeld HM (1961) Submicroscopic morphology and function of glial cells. *Int Rev Neurobiol* **3**:1–65.
- de Vries HE and Dijkstra DD (2004) Mononuclear phagocytes at the blood-brain barrier in multiple sclerosis, in *Blood-Spinal Cord and Brain Barriers in Health and Disease* (Sharma HS and Westman J eds) pp 409–417, Elsevier, San Diego.
- Dejana E, Lampugnani MG, Martinez-Estrada O, and Bazzoni G (2000) The molecular organization of endothelial junctions and their functional role in vascular morphogenesis and permeability. *Int J Dev Biol* **44**:743–748.
- Del Maschio A, De Luigi A, Martin-Padura I, Brockhaus M, Bartfai T, Fruscella P, Adorini L, Martino G, Furlan R, De Simoni MG, et al. (1999) Leukocyte recruitment in the cerebrospinal fluid of mice with experimental meningitis is inhibited by an antibody to junctional adhesion molecule (JAM). *J Exp Med* **190**:1351–1356.
- del Zoppo GJ and Hallenbeck JM (2000) Advances in the vascular pathophysiology of ischemic stroke. *Thromb Res* **98**:73–81.
- Dobbing J (1961) The blood-brain barrier. *Physiol Rev* **41**:130–188.
- Dore-Duffy P, Owen C, Balabanov R, Murphy S, Beaumont T, and Rafols JA (2000) Pericyte migration from the vascular wall in response to traumatic brain injury. *Microvasc Res* **60**:55–69.
- Duelli R, Staudt R, Grunwald F, and Kuschinsky W (1998a) Increase of glucose transporter densities (Glut1 and Glut3) during chronic administration of nicotine in rat brain. *Brain Res* **782**:36–42.
- Duelli R, Staudt R, Maurer MH, and Kuschinsky W (1998b) Local transport kinetics of glucose during acute and chronic nicotine infusion in rat brains. *J Neural Transm* **105**:1017–1028.
- Ehrlich P (1885) Das sauerstoffbedürfnis des organismus, in *Eine Farbenanalytische Studie*, Hirschwald, Berlin.
- Ehrlich P (1904) Ueber die beziehungen von chemischer constitution, verteilung und pharmakologischer wirkung, in *Gesammelte Arbeiten zur Immunitätsforschung*, pp 574, Hirschwald, Berlin.
- Fabian G, Szabo CA, Bozo B, Greenwood J, Adamson P, Deli MA, Joo F, Krizhai IA, and Szucs M (1998) Expression of G-protein subtypes in cultured cerebral endothelial cells. *Neurochem Int* **33**:179–185.
- Fanning AS, Jameson BJ, Jesaitis LA, and Anderson JM (1998) The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. *J Biol Chem* **273**:29745–29753.
- Farkas E and Luiten PG (2001) Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog Neurobiol* **64**:575–611.
- Farshori P and Kachar B (1999) Redistribution and phosphorylation of occludin during opening and resealing of tight junctions in cultured epithelial cells. *J Membr Biol* **170**:147–156.
- Fenstermacher J, Gross P, Sposito N, Acuff V, Pettersen S, and Gruber K (1988) Structural and functional variations in capillary systems within the brain. *Ann NY Acad Sci* **529**:21–30.
- Fiala M, Gan XH, Zhang L, House SD, Newton T, Graves MC, Shapshak P, Stins M, Kim KS, Witte M, et al. (1998) Cocaine enhances monocyte migration across the blood-brain barrier. Cocaine's connection to AIDS dementia and vasculitis? *Adv Exp Med Biol* **437**:199–205.
- Fiala M, Liu QN, Sayre J, Pop V, Brahmamand V, Graves MC, and Vinters H V (2002) Cyclooxygenase-2-positive macrophages infiltrate the Alzheimer's disease brain and damage the blood-brain barrier. *Eur J Clin Invest* **32**:360–371.
- Fischer S, Clauss M, Wiesnet M, Renz D, Schaper W, and Karliczek GF (1999) Hypoxia induces permeability in brain microvessel endothelial cells via VEGF and NO. *Am J Physiol* **276**:C812–C820.
- Fischer S, Wiesnet M, Marti HH, Renz D, and Schaper W (2004) Simultaneous activation of several second messengers in hypoxia-induced hyperpermeability of brain derived endothelial cells. *J Cell Physiol* **198**:359–369.
- Fischer S, Wobben M, Kleinstück J, Renz D, and Schaper W (2000) Effect of astroglial cells on hypoxia-induced permeability in PBMEC cells. *Am J Physiol Cell Physiol* **279**:C935–C944.
- Fischer S, Wobben M, Marti HH, Renz D, and Schaper W (2002) Hypoxia-induced hyperpermeability in brain microvessel endothelial cells involves VEGF-mediated changes in the expression of zonula occludens-1. *Microvasc Res* **63**:70–80.
- Friedemann U (1942) Blood-brain barrier. *Physiol Rev* **22**:125–145.
- Furuse M, Fujita K, Hiragi T, Fujimoto K, and Tsukita S (1998) Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J Cell Biol* **141**:1539–1550.
- Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, and Tsukita S (1993) Occludin: a novel integral membrane protein localizing at tight junctions. *J Cell Biol* **123**:1777–1788.
- Furuse M, Itoh M, Hirase T, Nagafuchi A, Yonemura S, and Tsukita S (1994) Direct association of occludin with ZO-1 and its possible involvement in the localization of occludin at tight junctions. *J Cell Biol* **127**:1617–1626.
- Furuse M, Sasaki H, and Tsukita S (1999) Manner of interaction of heterogeneous claudin species within and between tight junction strands. *J Cell Biol* **147**:891–903.
- Gan X, Zhang L, Berger O, Stins MF, Way D, Taub DD, Chang SL, Kim KS, House SD, Weinand M, Witte M, Graves MC and Fiala, M (1999) Cocaine enhances brain endothelial adhesion molecules and leukocyte migration. *Clin Immunol* **91**:68–76.
- Goldmann EE (1913) Vitalfarbung am zentralnervensystem. *Abhandl Konigl preuss Akad Wiss* **1**:1–60.
- Goldstein LB, Adams R, Becker K, Furberg CD, Gorelick PB, Hademenos G, Hill M, Howard G, Howard VJ, Jacobs B, et al. (2001) Primary prevention of ischemic stroke: a statement for healthcare professionals from the Stroke Council of the American Heart Association. *Stroke* **32**:280–299.
- Gonul E, Duz B, Kahraman S, Kayali H, Kubar A, and Timurkaynak E (2002) Early pericyte response to brain hypoxia in cats: an ultrastructural study. *Microvasc Res* **64**:116–119.
- Gonzalez-Mariscal L, Betanzos A, and Avila-Flores A (2000) MAGUK proteins: structure and role in the tight junction. *Semin Cell Dev Biol* **11**:315–324.
- Gottardi CJ, Arpin M, Fanning AS, and Louvard D (1996) The junction-associated protein, zonula occludens-1, localizes to the nucleus before the maturation and during the remodeling of cell-cell contacts. *Proc Natl Acad Sci USA* **93**:10779–10784.
- Gumbiner B, Lowenkopf T, and Apatira D (1991) Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1. *Proc Natl Acad Sci USA* **88**:3460–3464.
- Gursoy-Ozdemir Y, Qiu J, Matsuoka N, Bolay H, Bempohl D, Jin H, Wang X, Rosenberg GA, Lo EH, and Moskowitz MA (2004) Cortical spreading depression activates and upregulates MMP-9. *J Clin Invest* **113**:1447–1455.
- Hasegawa H, Fujita H, Katoh H, Aoki J, Nakamura K, Ichikawa A, and Negishi M (1999) Opposite regulation of transepithelial electrical resistance and paracellular permeability by Rho in Madin-Darby canine kidney cells. *J Biol Chem* **274**:20982–20988.
- Hatashita S and Hoff JT (1990) Brain edema and cerebrovascular permeability during cerebral ischemia in rats. *Stroke* **21**:582–588.
- Hawkins BT, Abruscato TJ, Egleton RD, Brown RC, Huber JD, Campos CR, and Davis TP (2004) Nicotine increases in vivo blood-brain barrier permeability and alters cerebral microvascular tight junction protein distribution. *Brain Res* **1027**:48–58.
- Hawkins BT, Brown RC, and Davis TP (2002) Smoking and ischemic stroke: a role for nicotine? *Trends Pharmacol Sci* **23**:78–82.
- Hayashi K, Nakao S, Nakaoka R, Nakagawa S, Kitagawa N, and Niwa M (2004) Effects of hypoxia on endothelial/pericyte co-culture model of the blood-brain barrier. *Regul Pept* **123**:77–83.
- Heiskala M, Peterson PA, and Yang Y (2001) The roles of claudin superfamily proteins in paracellular transport. *Traffic* **2**:93–98.
- Hirabayashi S, Tajima M, Yao I, Nishimura W, Mori H, and Hata Y (2003) JAM4, a junctional cell adhesion molecule interacting with a tight junction protein, MAGI-1. *Mol Cell Biol* **23**:4267–4282.
- Hirase T, Kawashima S, Wong EY, Ueyama T, Rikitake Y, Tsukita S, Yokoyama M, and Staddon JM (2001) Regulation of tight junction permeability and occludin phosphorylation by RhoA-p160ROCK-dependent and -independent mechanisms. *J Biol Chem* **276**:10423–10431.
- Hirase T, Staddon JM, Saitou M, Ando-Akatsuka Y, Itoh M, Furuse M, Fujimoto K, Tsukita S, and Rubin LL (1997) Occludin as a possible determinant of tight junction permeability in endothelial cells. *J Cell Sci* **110**:1603–1613.
- Hodgkin AL (1951) Ionic basis of electrical activity in nerve and muscle. *Biol Rev Cambridge Phil Soc* **26**:399–409.
- Hoffman HJ and Olszewski J (1961) Spread of sodium fluorescein in normal brain tissue. A study of the mechanism of the blood-brain barrier. *Neurology* **11**:1081–1085.
- Holash JA, Noden DM, and Stewart PA (1993) Re-evaluating the role of astrocytes in blood-brain barrier induction. *Dev Dyn* **197**:14–25.
- Hopkins AM, Li D, Mrsny RJ, Walsh SV, and Nusrat A (2000) Modulation of tight junction function by G protein-coupled events. *Adv Drug Deliv Rev* **41**:329–340.
- Hori S, Ohtsuki S, Hosoya K, Nakashima E, and Terasaki T (2004) A pericyte-derived angiotensin-1 multimeric complex induces occludin gene expression in brain capillary endothelial cells through Tie-2 activation in vitro. *J Neurochem* **89**:503–513.
- Howarth AG, Hughes MR, and Stevenson BR (1992) Detection of the tight junction-associated protein ZO-1 in astrocytes and other nonepithelial cell types. *Am J Physiol* **262**(2 Pt 1):C461–9.
- Huber JD, Egleton RD, and Davis TP (2001a) Molecular physiology and pathophysiology of tight junctions in the blood-brain barrier. *Trends Neurosci* **24**:719–725.
- Huber JD, Hau VS, Borg L, Campos CR, Egleton RD, and Davis TP (2002) Blood-brain barrier tight junctions are altered during a 72-h exposure to lambda-carrageenan-induced inflammatory pain. *Am J Physiol Heart Circ Physiol* **283**:H1531–H1537.
- Huber JD, Witt KA, Hom S, Egleton RD, Mark KS, and Davis TP (2001b) Inflammatory pain alters blood-brain barrier permeability and tight junctional protein expression. *Am J Physiol Heart Circ Physiol* **280**:H1241–H1248.
- Hynes RO (1992) Integrins: versatility, modulation and signaling in cell adhesion. *Cell* **69**:11–25.
- Ilecka J (1996) The structure and function of blood-brain barrier in ischaemic brain stroke process. *Ann Univ Mariae Curie Sklodowska* **51**:123–127.
- Inoko A, Itoh M, Tamura A, Matsuda M, Furuse M, and Tsukita S (2003) Expression and distribution of ZO-3, a tight junction MAGUK protein, in mouse tissues. *Genes Cells* **8**:837–845.
- Ishizaki T, Chiba H, Kojima T, Fujibe M, Soma T, Miyajima H, Nagasawa K, Wada I, and Sawada N (2003) Cyclic AMP induces phosphorylation of claudin-5 immunoprecipitates and expression of claudin-5 gene in blood-brain-barrier endothelial cells via protein kinase A-dependent and -independent pathways. *Exp Cell Res* **290**:275–288.
- Islas S, Vega J, Ponce L, and Gonzalez-Mariscal L (2002) Nuclear localization of the tight junction protein ZO-2 in epithelial cells. *Exp Cell Res* **274**:138–148.
- Itoh M, Morita K, and Tsukita S (1999) Characterization of ZO-2 as a MAGUK family member associated with tight as well as adherens junctions with a binding affinity to occludin and alpha catenin. *J Biol Chem* **274**:5981–5986.
- Itoh M, Nagafuchi A, Yonemura S, Kitani-Yasuda T, and Tsukita S (1993) The ZO-2 protein colocalizing with cadherins in non-epithelial cells is identical to ZO-1, a tight junction-associated protein in epithelial cells: cDNA cloning and immunoelectron microscopy. *J Cell Biol* **121**:491–502.

- Janzer RC and Raff MC (1987) Astrocytes induce blood-brain barrier properties in endothelial cells. *Nature (Lond)* **325**:253–257.
- Jensen AM and Westerfield M (2004) Zebrafish mosaic eyes is a novel FERM protein required for retinal lamination and retinal pigmented epithelial tight junction formation. *Curr Biol* **14**:711–717.
- Kale G, Naren AP, Sheth P, and Rao RK (2003) Tyrosine phosphorylation of occludin attenuates its interactions with ZO-1, ZO-2 and ZO-3. *Biochem Biophys Res Commun* **302**:324–329.
- Kempki O (2001) Cerebral edema. *Semin Nephrol* **21**:303–307.
- Kirk J, Plumb J, Mirakhor M, and McQuaid S (2003) Tight junctional abnormality in multiple sclerosis white matter affects all calibres of vessel and is associated with blood-brain barrier leakage and active demyelination. *J Pathol* **201**:319–327.
- Klingler C, Kniesel U, Bamforth SD, Wolburg H, Engelhardt B, and Risau W (2000) Disruption of epithelial tight junctions is prevented by cyclic nucleotide-dependent protein kinase inhibitors. *Histochem Cell Biol* **113**:349–361.
- Kniesel U and Wolburg H (2000) Tight junctions of the blood-brain barrier. *Cell Mol Neurobiol* **20**:57–76.
- Knudsen KA, Soler AP, Johnson KR, and Wheelock MJ (1995) Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alpha-catenin. *J Cell Biol* **130**:67–77.
- Kobayashi H, Magnoni MS, Govoni S, Izumi F, Wada A, and Trabucchi M (1985) Neuronal control of brain microvessel function. *Experientia* **41**:427–434.
- Kojima T, Yamamoto T, Murata M, Chiba H, Kokai Y, and Sawada N (2003) Regulation of the blood-biliary barrier: interaction between gap and tight junctions in hepatocytes. *Med Electron Microsc* **36**:157–164.
- Kondo T, Kinouchi H, Kawase M, and Yoshimoto T (1996) Astroglial cells inhibit the increasing permeability of brain endothelial cell monolayer following hypoxia/reoxygenation. *Neurosci Lett* **208**:101–104.
- Krum JM, Kenyon KL, and Rosenztein JM (1997) Expression of blood-brain barrier characteristics following neuronal loss and astroglial damage after administration of anti-Thy-1 immunotoxin. *Exp Neurol* **146**:33–45.
- Kubota K, Furuse M, Sasaki H, Sonoda N, Fujita K, Nagafuchi A, and Tsukita S (1999) Ca(2+)-independent cell-adhesion activity of claudins, a family of integral membrane proteins localized at tight junctions. *Curr Biol* **9**:1035–1038.
- Kurihara H, Anderson JM, and Farquhar MG (1995) Increased Tyr phosphorylation of ZO-1 during modification of tight junctions between glomerular foot processes. *Am J Physiol* **268**:F514–F524.
- Lampugnani MG, Corada M, Caveda L, Breviario F, Ayalon O, Geiger B, and Dejana E (1995) The molecular organization of endothelial cell to cell junctions: differential association of plakoglobin, beta-catenin and alpha-catenin with vascular endothelial cadherin (VE-cadherin). *J Cell Biol* **129**:203–217.
- Le Douarin N (1973) A biological cell labeling technique and its use in experimental embryology. *Dev Biol* **30**:217–222.
- Lee EJ, Hung YC, and Lee MY (1999) Early alterations in cerebral hemodynamics, brain metabolism and blood-brain barrier permeability in experimental intracerebral hemorrhage. *J Neurosurg* **91**:1013–1019.
- Lewandowsky M (1900) Zur lehre von der cerebrospinalflussigkeit. *Z Klin Med* **40**:480–494.
- Lippoldt A, Kniesel U, Liebner S, Kalbacher H, Kirsch T, Wolburg H, and Haller H (2000) Structural alterations of tight junctions are associated with loss of polarity in stroke-prone spontaneously hypertensive rat blood-brain barrier endothelial cells. *Brain Res* **885**:251–261.
- Lo EH, Broderick JP, and Moskowitz MA (2004) tPA and proteolysis in the neurovascular unit. *Stroke* **35**:354–356.
- Mark KS, Burroughs AR, Brown RC, Huber JD, and Davis TP (2004) Nitric oxide mediates hypoxia-induced changes in paracellular permeability of cerebral microvasculature. *Am J Physiol Heart Circ Physiol* **286**:H174–H178.
- Mark KS and Davis TP (2002) Cerebral microvascular changes in permeability and tight junctions induced by hypoxia-reoxygenation. *Am J Physiol Heart Circ Physiol* **282**:H1485–H1494.
- Martin-Padura I, Lostaglio S, Schneemann M, Williams L, Romano M, Fruscella P, Panzeri C, Stoppacciaro A, Ruco L, Villa A, et al. (1998) Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. *J Cell Biol* **142**:117–127.
- Mattagajasingh SN, Huang SC, Hartenstein JS, and Benz EJ Jr (2000) Characterization of the interaction between protein 4.1R and ZO-2. A possible link between the tight junction and the actin cytoskeleton. *J Biol Chem* **275**:30573–30585.
- Maxwell K, Berliner JA, and Canciani PA (1987) Induction of gamma-glutamyl transpeptidase in cultured cerebral endothelial cells by a product released by astrocytes. *Brain Res* **410**:309–314.
- Maynard EA, Schultz RL, and Pease DC (1957) Electron microscopy of the vascular bed of rat cerebral cortex. *Am J Anat* **100**:409–433.
- McCarthy KM, Skare IB, Stankewich MC, Furuse M, Tsukita S, Rogers RA, Lynch RD, and Schneeberger EE (1996) Occludin is a functional component of the tight junction. *J Cell Sci* **109**:2287–2298.
- McQuaid S, Plumb J, Mirakhor M, and Kirk J (2002) Abnormal endothelial tight junctions in active lesions and normal-appearing white matter in multiple sclerosis. *Neuropathol Appl Neurobiol* **28**:153.
- Meyer TN, Schwesinger C, and Denker BM (2002) Zonula occludens-1 is a scaffolding protein for signaling molecules. Galpha(12) directly binds to the SH3 domain and regulates paracellular permeability in epithelial cells. *J Biol Chem* **277**:2121.
- Minagar A and Alexander JS (2003) Blood-brain barrier disruption in multiple sclerosis. *Mult Scler* **9**:540–549.
- Morcus Y, Hsieh MJ, Bauer HC, and Chan-Ling T (2001) Immunolocalization of occludin and claudin-1 to tight junctions in intact CNS vessels of mammalian retina. *J Neurocytol* **30**:107–123.
- Morganti-Kossmann MC, Rancan M, Stahel PF, and Kossmann T (2002) Inflammatory response in acute traumatic brain injury: a double-edged sword. *Curr Opin Crit Care* **8**:101–105.
- Morrissey SP, Stodal H, Zettl U, Simonis C, Jung S, Kiefer R, Lassmann H, Hartung HP, Haase A, and Toyka KV (1996) In vivo MRI and its histological correlates in acute adoptive transfer experimental allergic encephalomyelitis. Quantification of inflammation and oedema. *Brain* **119**:239–248.
- Nagy Z, Goehrlert UG, Wolfe LS, and Huttner I (1985) Ca²⁺ depletion-induced disconnection of tight junctions in isolated rat brain microvessels. *Acta Neuropathol (Berl)* **68**:48–52.
- Nath A, Maragos WF, Avison MJ, Schmitt FA, and Berger JR (2001) Acceleration of HIV dementia with methamphetamine and cocaine. *J Neuroviral* **7**:66–71.
- Neuhaus J, Risau W, and Wolburg H (1991) Induction of blood-brain barrier characteristics in bovine brain endothelial cells by rat astroglial cells in transfilter coculture. *Ann NY Acad Sci* **633**:578–580.
- Neuwelt EA (2004) Mechanisms of disease: the blood-brain barrier. *Neurosurgery* **54**:131–140.
- Nishimura M, Kakizaki M, Ono Y, Morimoto K, Takeuchi M, Inoue Y, Imai T, and Takai Y (2002) JEAP, a novel component of tight junctions in exocrine cells. *J Biol Chem* **277**:5583–5587.
- Nitta T, Hata M, Gotoh S, Seo Y, Sasaki H, Hashimoto N, Furuse M, and Tsukita S (2003) Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. *J Cell Biol* **161**:653–660.
- Ohnishi H, Nakahara T, Furuse K, Sasaki H, Tsukita S, and Furuse M (2004) JACOP: a novel plaque protein localizing at the apical junctional complex with sequence similarity to cingulin. *J Biol Chem*.
- Oldendorf WH, Cornford ME, and Brown WJ (1977) The large apparent work capability of the blood-brain barrier: a study of the mitochondrial content of capillary endothelial cells in brain and other tissues of the rat. *Ann Neurol* **1**:409–417.
- Oztas B, Akgul S, and Arslan FB (2004) Influence of surgical pain stress on the blood-brain barrier permeability in rats. *Life Sci* **74**:1973–1979.
- Paemeleire K (2002) The cellular basis of neurovascular metabolic coupling. *Acta Neurol Belg* **102**:153–157.
- Papadopoulos MC, Saadoun S, Binder DK, Manley GT, Krishna S, and Verkman AS (2004) Molecular mechanisms of brain tumor edema. *Neuroscience* **129**:1011–1020.
- Papadopoulos MC, Saadoun S, Woodrow CJ, Davies DC, Costa-Martins P, Moss RF, Krishna S, and Bell BA (2001) Occludin expression in microvessels of neoplastic and non-neoplastic human brain. *Neuropathol Appl Neurobiol* **27**:384–395.
- Petty MA and Lo EH (2002) Junctional complexes of the blood-brain barrier: permeability changes in neuroinflammation. *Prog Neurobiol* **68**:311–323.
- Petty MA and Wettstein JG (2001) Elements of cerebral microvascular ischaemia. *Brain Res Brain Res Rev* **36**:23–34.
- Plateel M, Teissier E, and Cecchelli R (1997) Hypoxia dramatically increases the nonspecific transport of blood-borne proteins to the brain. *J Neurochem* **68**:874–877.
- Ramsauer M, Krause D, and Dermietzel R (2002) Angiogenesis of the blood-brain barrier in vitro and the function of cerebral pericytes. *FASEB J* **16**:1274–1276.
- Rao RK, Basuroy S, Rao VU, Karnaky KJ Jr, and Gupta A (2002) Tyrosine phosphorylation and dissociation of occludin-ZO-1 and E-cadherin-beta-catenin complexes from the cytoskeleton by oxidative stress. *Biochem J* **368**:471–481.
- Rascher G, Fischmann A, Kroger S, Duffner F, Grote EH, and Wolburg H (2002) Extracellular matrix and the blood-brain barrier in glioblastoma multiforme: spatial segregation of tenascin and agrin. *Acta Neuropathol (Berl)* **104**:85–91.
- Raschperger E, Engstrom U, Pettersson RF, and Fuxe J (2004) CLMP, a novel member of the CTX family and a new component of epithelial tight junctions. *J Biol Chem* **279**:796–804.
- Reese TS and Karnovsky MJ (1967) Fine structural localization of a blood-brain barrier to exogenous peroxidase. *J Cell Biol* **34**:207–217.
- Riesen FK, Rothen-Rutishauser B, and Wunderli-Allenspach H (2002) A ZO1-GFP fusion protein to study the dynamics of tight junctions in living cells. *Histochem Cell Biol* **117**:307–315.
- Rolfe DF and Brown GC (1997) Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol Rev* **77**:731–758.
- Romero IA, Radewicz K, Jubin E, Michel CC, Greenwood J, Couraud PO, and Adamson P (2003) Changes in cytoskeletal and tight junctional proteins correlate with decreased permeability induced by dexamethasone in cultured rat brain endothelial cells. *Neurosci Lett* **344**:112–116.
- Rosenberg GA, Estrada E, Kelley RO, and Kornfeld M (1993) Bacterial collagenase disrupts extracellular matrix and opens blood-brain barrier in rat. *Neurosci Lett* **160**:117–119.
- Saha C, Nigam SK, and Denker BM (1998) Involvement of Galpha2 in the maintenance and biogenesis of epithelial cell tight junctions. *J Biol Chem* **273**:21629–21633.
- Sakakibara A, Furuse M, Saitou M, Ando-Akatsuka Y, and Tsukita S (1997) Possible involvement of phosphorylation of occludin in tight junction formation. *J Cell Biol* **137**:1393–1401.
- Sanchez-del-Rio M and Reuter U (2004) Migraine aura: new information on underlying mechanisms. *Curr Opin Neurol* **17**:289–293.
- Savettieri G, Di Liegro I, Catania C, Licata L, Pitarresi GL, D'Agostino S, Schiera G, De Caro V, Giandalia G, Giannola LI, et al. (2000) Neurons and ECM regulate occludin localization in brain endothelial cells. *Neuroreport* **11**:1081–1084.
- Schulze C and Firth J (1993) A immunohistochemical localization of adherens junction components in blood-brain barrier microvessels of the rat. *J Cell Sci* **104**:773–782.
- Schulze C, Smales C, Rubin LL, and Staddon JM (1997) Lysophosphatidic acid increases tight junction permeability in cultured brain endothelial cells. *J Neurochem* **68**:991–1000.
- Sedlakova R, Shivers RR, and Del Maestro RF (1999) Ultrastructure of the blood-brain barrier in the rabbit. *J Submicrosc Cytol Pathol* **31**:149–161.
- Seeldrayers PA, Syha J, Morrissey SP, Stodal H, Vass K, Jung S, Gneiting T, Lassmann H, Haase A, and Hartung HP (1993) Magnetic resonance imaging investigation of blood-brain barrier damage in adoptive transfer experimental autoimmune encephalomyelitis. *J Neuroimmunol* **46**:199–206.
- Shin H, Hsueh YP, Yang FC, Kim E, and Sheng M (2000) An intramolecular

- interaction between Src homology 3 domain and guanylate kinase-like domain required for channel clustering by postsynaptic density-95/SAP90. *J Neurosci* **20**:3580–3587.
- Simard M, Arcuino G, Takano T, Liu QS, and Nedergaard M (2003) Signaling at the gliovascular interface. *J Neurosci* **23**:9254–9262.
- Song L and Pachter JS (2004) Monocyte chemoattractant protein-1 alters expression of tight junction-associated proteins in brain microvascular endothelial cells. *Microvasc Res* **67**:78–89.
- Staddon JM, Herrenknecht K, Smales C, and Rubin LL (1995) Evidence that tyrosine phosphorylation may increase tight junction permeability. *J Cell Sci* **108**:609–619.
- Stevenson BR, Anderson JM, Braun ID, and Mooseker MS (1989) Phosphorylation of the tight-junction protein ZO-1 in two strains of Madin-Darby canine kidney cells which differ in transepithelial resistance. *Biochem J* **263**:597–599.
- Stevenson BR and Keon BH (1998) The tight junction: morphology to molecules. *Annu Rev Cell Dev Biol* **14**:89–109.
- Stevenson BR, Siliciano JD, Mooseker MS, and Goodenough DA (1986) Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. *J Cell Biol* **103**:755–766.
- Stewart PA and Wiley MJ (1981) Developing nervous tissue induces formation of blood-brain barrier characteristics in invading endothelial cells: a study using quail-chick transplantation chimeras. *Dev Biol* **84**:183–192.
- Stuart RO, Sun A, Bush KT, and Nigam SK (1996) Dependence of epithelial intercellular junction biogenesis on thapsigargin-sensitive intracellular calcium stores. *J Biol Chem* **271**:13636–13641.
- Tagami M, Nara Y, Kubota A, Fujino H, and Yamori Y (1990) Ultrastructural changes in cerebral pericytes and astrocytes of stroke-prone spontaneously hypertensive rats. *Stroke* **21**:1064–1071.
- Tao-Cheng JH, Nagy Z, and Brightman MW (1987) Tight junctions of brain endothelium in vitro are enhanced by astroglia. *J Neurosci* **7**(10):3293–3299.
- Tilling T, Engelbertz C, Decker S, Korte D, Huwel S, and Galla HJ (2002) Expression and adhesive properties of basement membrane proteins in cerebral capillary endothelial cell cultures. *Cell Tissue Res* **310**:19–29.
- Tilling T, Korte D, Hoheisel D, and Galla HJ (1998) Basement membrane proteins influence brain capillary endothelial barrier function in vitro. *J Neurochem* **71**:1151–1157.
- Tong XK and Hamel E (1999) Regional cholinergic denervation of cortical microvessels and nitric oxide synthase-containing neurons in Alzheimer's disease. *Neuroscience* **92**:163–175.
- Toyofuku T, Yabuki M, Otsu K, Kuzuya T, Hori M, and Tada M (1998) Direct association of the gap junction protein connexin-43 with ZO-1 in cardiac myocytes. *J Biol Chem* **273**:12725–12731.
- Traweger A, Fuchs R, Krizbai IA, Weiger TM, Bauer HC, and Bauer H (2002) The tight junction protein ZO-2 localizes to the nucleus and interacts with the heterogeneous nuclear ribonucleoprotein scaffold attachment factor-B. *J Biol Chem* **278**:2692–2700.
- Tschirgi RD (1962) Blood-brain barrier: fact or fancy? *Fed Proc* **21**:665–671.
- Tsukamoto T and Nigam SK (1999) Role of tyrosine phosphorylation in the reassembly of occludin and other tight junction proteins. *Am J Physiol* **276**:F737–F750.
- Umeda K, Matsui T, Nakayama M, Furuse K, Sasaki H, Furuse M, and Tsukita S (2004) Establishment and characterization of cultured epithelial cells lacking expression of ZO-1. *J Biol Chem* **279**:44785–44794.
- van Bruggen N, Thibodeaux H, Palmer JT, Lee WP, Fu L, Cairns B, Tumas D, Gerlai R, Williams SP, van Lookeren Campagne M, et al. (1999) VEGF antagonism reduces edema formation and tissue damage after ischemia/reperfusion injury in the mouse brain. *J Clin Invest* **104**:1613–1620.
- Vaucher E and Hamel E (1995) Cholinergic basal forebrain neurons project to cortical microvessels in the rat: electron microscopic study with anterogradely transported Phaseolus vulgaris leucoagglutinin and choline acetyltransferase immunocytochemistry. *J Neurosci* **15**:7427–7441.
- Vaucher E, Tong XK, Cholet N, Lantin S, and Hamel E (2000) GABA neurons provide a rich input to microvessels but not nitric oxide neurons in the rat cerebral cortex: a means for direct regulation of local cerebral blood flow. *J Comp Neurol* **421**:161–171.
- Vincent PA, Xiao K, Buckley KM, and Kowalczyk AP (2004) VE-cadherin: adhesion at arm's length. *Am J Physiol Cell Physiol* **286**:C987–C997.
- Vorbrodt AW and Dobrogowska DH (2003) Molecular anatomy of intercellular junctions in brain endothelial and epithelial barriers: electron microscopist's view. *Brain Res Brain Res Rev* **42**:221–242.
- Wachtel M, Frei K, Ehler E, Fontana A, Winterhalter K and Gloor SM (1999) Occludin proteolysis and increased permeability in endothelial cells through tyrosine phosphatase inhibition. *J Cell Sci* **112**:4347–4356.
- Wang L, McComb JG, Weiss MH, McDonough AA, and Zlokovic BV (1994) Nicotine downregulates alpha 2 isoform of Na,K-ATPase at the blood-brain barrier and brain in rats. *Biochem Biophys Res Commun* **199**:1422–1427.
- Wang X, Tsuji K, Lee SR, Ning M, Furie KL, Buchan AM, and Lo EH (2004) Mechanisms of hemorrhagic transformation after tissue plasminogen activator reperfusion therapy for ischemic stroke. *Stroke* **35** (Suppl 1):2726–2730.
- Wardlaw JM, Sandercock PA, Dennis MS, and Starr J (2003) Is breakdown of the blood-brain barrier responsible for lacunar stroke, leukoaraiosis and dementia? *Stroke* **34**:806–812.
- Watabe-Uchida M, Uchida N, Imamura Y, Nagafuchi A, Fujimoto K, Uemura T, Vermeulen S, van Roy F, Adamson ED, and Takeichi M (1998) Alpha-catenin-vinculin interaction functions to organize the apical junctional complex in epithelial cells. *J Cell Biol* **142**:847–857.
- Willis CL, Nolan CC, Reith SN, Lister T, Prior MJ, Guerin CJ, Mavroudis G, and Ray DE (2004) Focal astrocyte loss is followed by microvascular damage, with subsequent repair of the blood-brain barrier in the apparent absence of direct astrocyte contact. *Glia* **45**:325–337.
- Witt KA, Mark KS, Hom S, and Davis TP (2003) Effects of hypoxia-reoxygenation on rat blood-brain barrier permeability and tight junctional protein expression. *Am J Physiol Heart Circ Physiol* **285**:H2820–H2831.
- Witt KA, Mark KS, Huber J, and Davis TP (2005) Hypoxia-inducible factor and nuclear factor kappa-B activation in blood-brain barrier endothelium under hypoxic/reoxygenation stress. *J Neurochem* **92**:203–214.
- Wolburg H and Lippoldt A (2002) Tight junctions of the blood-brain barrier: development, composition and regulation. *Vascul Pharmacol* **38**:323–337.
- Wolburg H, Wolburg-Buchholz K, Kraus J, Rascher-Eggstein G, Liebner S, Hamm S, Duffner F, Grote EH, Risau W, and Engelhardt B (2003) Localization of claudin-3 in tight junctions of the blood-brain barrier is selectively lost during experimental autoimmune encephalomyelitis and human glioblastoma multiforme. *Acta Neuropathol* **105**:586–592.
- Wong V and Gumbiner BM (1997) A synthetic peptide corresponding to the extracellular domain of occludin perturbs the tight junction permeability barrier. *J Cell Biol* **136**:399–409.
- Yamamoto T, Harada N, Kawano Y, Taya S, and Kaibuchi K (1999) In vivo interaction of AF-6 with activated Ras and ZO-1. *Biochem Biophys Res Commun* **259**:103–107.
- Ye J, Tsukamoto T, Sun A, and Nigam SK (1999) A role for intracellular calcium in tight junction reassembly after ATP depletion-repletion. *Am J Physiol* **277**:F524–F532.
- Zhang L, Looney D, Taub D, Chang SL, Way D, Witte MH, Graves MC, and Fiala M (1998) Cocaine opens the blood-brain barrier to HIV-1 invasion. *J Neurovirol* **4**:619–626.
- Zhong Y, Enomoto K, Tobioka H, Konishi Y, Satoh M, and Mori M (1994) Sequential decrease in tight junctions as revealed by 7H6 tight junction-associated protein during rat hepatocarcinogenesis. *Jpn J Cancer Res* **85**:351–356.
- Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T, and Carmignoto G (2003) Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat Neurosci* **6**:43–50.