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Review

Blood-neural Barrier: Intercellular Communication at Glio-Vascular Interface

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The blood-neural barrier (BNB), including blood-brain barrier (BBB) and blood-retinal barrier (BRB), is an endothelial barrier constructed by an extensive network of endothelial cells, astrocytes and neurons to form functional 'neurovascular units', which has an important role in maintaining a precisely regulated microenvironment for reliable neuronal activity. Although failure of the BNB may be a precipitating event or a consequence, the breakdown of BNB is closely related with the development and progression of CNS diseases. Therefore, BNB is most essential in the regulation of microenvironment of the CNS. The BNB is a selective diffusion barrier characterized by tight junctions between endothelial cells, lack of fenestrations, and specific BNB transporters. The BNB have been shown to be astrocyte dependent, for it is formed by the CNS capillary endothelial cells, surrounded by astrocytic end-foot processes. Given the anatomical associations with endothelial cells, it could be supposed that astrocytes play a role in the development, maintenance, and breakdown of the BNB. Therefore, astrocytes-endothelial cells interaction influences the BNB in both physiological and pathological conditions. If we better understand mutual interactions between astrocytes and endothelial cells, in the near future, we could provide a critical solution to the BNB problems and create new opportunities for future success of treating CNS diseases. Here, we focused astrocyte-endothelial cell interaction in the formation and function of the BNB.

Keywords: Astrocyte, Blood brain barrier, Blood neural barrier, Blood retinal barrier, Central nervous system, Endothelial cell

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Introduction

Central nervous system (CNS) is the most sensitive and critical system in all mammals. Therefore, it is necessary to have developed the specialized structure to isolate their neurons from blood or cerebrospinal fluid to maintain a stable ionic environment and to ensure appropriate activities of neurons. The interface between the CNS and the circulatory system (blood or cerebrospinal fluid) forms the barrier to regulate ionic balances, facilitate nutrient transport, and block potentially harmful molecules. That is a cellular barrier to represent the boundary between the CNS capillaries and the extracellular fluid of neuron and glial cells. There are three main barrier layers in the CNS: the endothelium of brain or retinal capillaries [blood-brain barrier (BBB) or blood-retinal barrier (BRB)], the choroids plexus epithelium (blood-cerebrospinal fluid barrier), and the arachnoid epithelium of the meninges (Abbott, 2004). Such barriers differ in localization, size, morphology, and function. For example, the BBB or BRB is an endothelial barrier, where tight junctions between the endothelial cells seal off vascular lumen. In contrast, blood-cerebrospinal fluid barrier is formed by the choroids plexus epithelium, where tight junctions between the epithelial cells are formed, but the capillaries are fenestrated. Individual neurons are almost within 8-20 μm from capillaries, whereas they sometimes exist millimeters or centimeters apart from cerebrospinal fluid (Schlageter *et al.*, 1999). The blood-neural barrier (BNB), including BBB and BRB, is a selective barrier formed by the CNS capillary endothelial cells which is typically surrounded by glial cell end-foot processes (Risau and Wolburg, 1990). Major characteristics of BNB have been shown to be astrocyte dependent, suggesting the close association between the glial-end foot processes and endothelial cells (Orte *et al.*,1999).

The breakdown of the BNB are closely related with

variable CNS diseases, including brain edema, stroke, ischemic retinopathies, diabetic retinopathy, retinopathy of prematurity, Alzheimer's disease, multiple sclerosis, and tumors of the CNS. Therefore, BNB is most essential in the regulation of microenvironment of the CNS. On the other hand, the BNB is the bottleneck in drug delivery to the CNS, which is the most important factor limiting neurotherapeutics. Most drugs can not cross the BNB. Despite of this importance of the BNB, little is known about the fundamental mechanisms of development, maintenance, and breakdown of the BNB. Therefore, it is an inevitable task to be solved that vascular biologists and neuroscientists have to provide critical keys for solutions to BBB problems.

In this article, astrocyte-endothelial cell interaction in the formation and function of the BNB is focused.

Which cells is the BNB composed of?

The BNB is a selective diffusion barrier characterized by tight junctions between endothelial cells, lack of fenestrations, and specific BNB transporters. Basically, general framework of the BNB was established at 1960's (Reese and Karnovsky, 1967; Brightman and Reese, 1969). The endothelial cells of the BNB are different from other endothelial cells by active metabolism with much mitochondrial content (Oldendorf *et al*., 1977), low number of pinocytotic vesicles (Sedlakova *et al*., 1999), lack of fenestration (Fenstermacher *et al*., 1988), and tight junctions between endothelial cells (Kniesel and Wolburg, 2000). Endothelial cells and pericytes are surrounded by a membrane composed of collagen type IV, laminin, fibronectin, and heparin sulfate proteoglycan (Farkas and Luiten, 2001), which is ensheathed by astrocyte end-foot processes (Fig. 1).

Endothelial cells. The endothelial cells of the CNS protect the CNS from the affection of changes in the vascular system, and serve the nutritional demands of the CNS. The BNB is located in the endothelial cells, which are sealed off by tight junctions between endothelial cells to serve as a physical blockade to paracellular diffusion of ions, peptides, and immune cells from the blood into the CNS (Huber *et al*., 2001) (Fig. 1). Almost 100% of large-molecules (>400 Da) do not cross the BNB. In addition, more than 98% of all small-molecules can not go through the BNB (Pardridge, 2005). Tight junctions are the most critical structure in the barrier function of BNB. Although tight junctions result from the triangular relationship among endothelial cells, glial cells, and neurons (Dermietzel and Krause, 1991), the most prominent characteristic of endothelial cells in the CNS is the tight junction. The tight junction complex includes two classes of transmembrane molecules - occludin and claudins - which interact with transmembrane proteins on adjacent endothelial cells, forming a physical barrier to paracellular diffusion

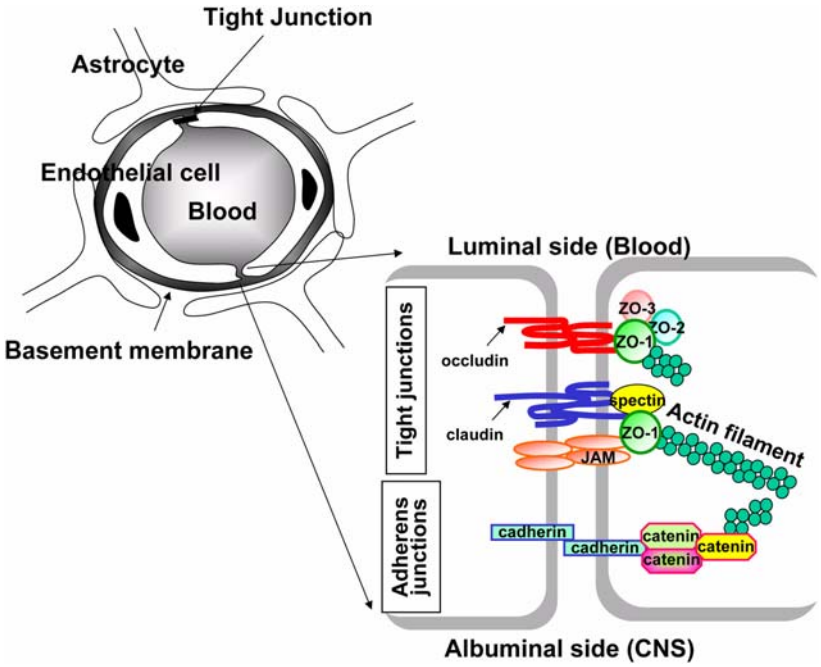

Fig. 1. Schematic representations of cross-section of CNS capillary and tight junction complex between endothelial cells. The Endothelial cells and pericytes are surrounded by a membrane composed of collagen type IV, laminin, fibronectin, and heparin sulfate proteoglycan, which is ensheathed by astrocyte end-foot processes. The tight junction complex includes two classes of transmembrane molecules (occludin and claudins), which interact with transmembrane proteins on adjacent endothelial cells. The cytoplasmic tails of the occludin–claudin complex are linked to the actin cytoskeleton via a number of accessory proteins, including members of the zonula occludens family, ZO-1, ZO-2, and ZO-3.

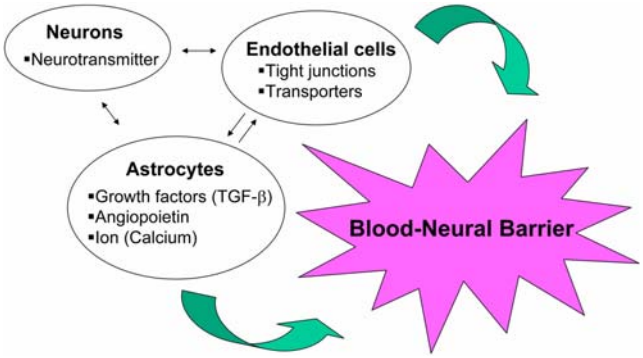

Fig. 2. Blood neural barrier (BNB) as a functional neurovascular unit. BNB is constructed by an extensive network of endothelial cells, astrocytes and neurons. Mutual interactions between each component contribute the formation, maintenance, and dysfunction of BNB.

(Hirase *et al*., 1997; Furuse *et al*., 1998). The cytoplasmic tails of the occludin-claudin complex are linked to the actin cytoskeleton via a number of accessory proteins, including members of the zonula occludens family, ZO-1, ZO-2, and ZO-3. These proteins are part of the membrane-associated guanylate kinase family (Anderson *et al*., 1995; Haskins *et al*., 1998), and contain multiple PDZ domains for association with signaling proteins (Haskins *et al*., 1998; Reichert *et al*., 2000). ZO-1 and ZO-2, the most widely studied members of the family, interact with both occludin and claudins, and the actin cytoskeleton (Fanning *et al*., 1998; Itoh *et al*., 1999), anchoring the transmembrane adhesion proteins to the cytoskeletal scaffold of the endothelial cell. The tight junction functions not only as a physical blockade, but also as a segregator of the apical and basal domains in the endothelium. The apical-basal polarity of endothelial cells is clearly reflected in the transporter systems (Wolburg, 2006).

The BNB has three types of transporters including carrier-mediated, active efflux, and receptor-mediated transporters. The former two are for the delivery of small molecules, and the last one is for large molecules. The endothelial cells in the CNS have transporters to supply neurons with nutrients including GLUT1 glucose carrier, LAT1 amino acid carrier, and transporters for nucleosides, nucleobases, and other substances (Begley and Brightman, 2003).

Astrocyte. Astrocytes had been considered to be the 'glue of CNS', which play a role as scaffolds in neuronal development and interactions. Recently, it has been revealed that astrocytes have functions such as the control of CNS vascular tone, neurogenesis, and synaptogenesis. With increasing knowledge of astrocyte function, it would be improved to understand the CNS physiology and its pathology including BNB formation, maintenance, and breakdown. However, until now the characterization of astrocytes at the single cell level is still inconclusive.

Astrocytes almost ensheath the endothelial cells and attached pericytes by end-foot processes (Kacem *et al*., 1998). In addition, they interconnect endothelial cells with surrounding neurons. Given the anatomical associations with endothelial cells, it could be supposed that astrocytes play a role in the development, maintenance, and breakdown of the BNB (Janzer and Raff, 1997). Endothelial cell lines can grow as a monolayer and retain some phenotypes of BNB. However, they lose important BNB characteristics such as loosening of tight junction and loss of transporter systems. Although inductive influences from astrocytes are critical, successful formation or maintenance of the BNB depends on the local conditions and maturational stages (Krum *et al*., 1997). There are some differences between species in the BNB tightening (Bauer and Bauer, 2000), and transporters maturation (Braun *et al*., 1980). Interestingly, astrocytes also can induce junctional tightening and BNB markers up-regulation even in the non-CNS endothelial cells (Hurst and Fritz, 1996; Kuchler-Bopp *et al*., 1999).

Mutual interactions between astrocytes, endothelial cells, and neurons are absolutely required. Although the specialized communication between astrocytes and endothelial cells or neurons is increasingly recognized, the comprehensive understanding of the structural and functional relationships is not yet possible.

Pericyte and microglia. Pericytes migrate into neuroepithelial cell layer during the angiogenesis of the developing CNS. Pericytes are in close proximity to endothelial cells, separated only by a shared basement membrane. Interactions between endothelial cells and pericytes are mediated by endothelin-1 (Dehouck *et al*., 1997). Little has been proven about the function of pericytes in the BNB. Periytes have contractile proteins to contract capillary to regulate blood flow (Bandopadhyay *et al*., 2001). Pericytes induce or maintain the BNB of endothelial cells in a manner similar to astrocytes (Hori *et al*., 2004). However pericytes are separated off in the hypoxia and trauma, which is associated with the BNB breakdown (Dore-Duffy *et al*., 2000; Gonul *et al*., 2002).

Microglias are originated from blood-borne cells, and comigrate with endothelial cells into the CNS, which is regarded as endogenous immune cells in the CNS. Although some functions like trapping foreign materials (Xu and Ling, 1994) or guiding monocytes across endothelial cells (Peridsky *et al*., 1999) are suggested, their role in the BNB remains to be elucidated.

Neuron. Blood vessels are closely linked to neuronal activity. Thus, blood flow increases in the response to local neuronal activation (Iadecola, 1993). However, the cellular mechanisms of this process are not completely understood (Leybaert, 2005). The breakdown of BNB is often followed by pathological changes of blood flow and perfusion pressure, which would be a compensatory mechanism rather than the simple anatomic disruption (Lee *et al*., 1999). In adult CNS, neurons are not directly contact with endothelial cells. Instead,

astrocytes mediate the neurovascular connection, whereas, during development, undifferentiated neurons can be contact with endothelial cells. Therefore, early neurons may influence the induction of the BNB in endothelial cells (Pardridge, 1999).

How astrocytes induce and maintain the BNB in endothelial cells?

Gliogenesis usually begins in late embryonic stage, and only during postnatal development, astrocytes become prominent. Therefore, astrocytes don't initially induce BNB formation. The molecular mechanisms of BNB induction are still unclear. Nevertheless, astrocytes play a critical role in BNB induction and maintenance. Actually, astrocytes can upregulate tight junction proteins (Dehouck *et al*., 1990), transporters like GLUT1 (McAllister, 2001) and Pgp (Schinkel, 1999), and barrier-related marker enzyme activities in enodothelial cells. These critical functions of astrocytes for BNB induction and maintenance are primarily due to 1) end-foot processes of astrocytes and 2) inducing factors from astrocytes (Fig. 2).

CNS capillaries are closely surrounded by end-foot processes of astrocytes, pericytes, microglia and neuronal processes. These close cell to cell contacts, especially astrocytes and endothelial cells, led to the suggestion that astrocytes could mediate induction of the BNB in endothelial cells (Davson and Oldendorf, 1967). It is also possible that endothelial cells induce the growth and differentiation of astrocytes (Mi *et al*., 2001). Astrocytic end-feet have specialized features of high density of orthogonal array of particles containing the water channel aquaporin 4, and the potassium channel Kir4.1. The polarity of orthogonal array of particles correlates with the expression of agrin on the basal lamina (Wolburg and Lippoldt, 2002). The precise localization of these particles in the astrocytic end-feet, anchored by agrin, contributes the inductive influences between astrocytes and endothelial cells.

Considering the interaction between neighboring cells such as endothelial cells and neurons, astrocytes secrete several astrocyte-derived factors associated with cell to cell interaction for BNB formation. Angiopoietin-1 (ang-1) seems partly to be responsible for this. Mice lacking or overexpressing ang-1 have revealed that ang-1 is responsible for recruiting and sustaining periendothelial support cells and for contributing to the impermeability of blood vessels (Thurston *et al*., 1999). The secretion of ang-1 from perivascular astrocytes can bind its receptor, Tie-2 onto endothelial cells. Recently, it has been shown that SSeCKS stimulate astrocytic expression and secretion of angiopoietin-1. SSeCKS-conditioned media increase the expression of tight junction proteins and decrease the permeability of endothelial cells (Lee *et al*., 2003). Exposure of astrocytes to oxygenation following hypoxia leads thrombospondin-1 (TSP-1) to increase and sustain for a while (Song *et al*., 2002). TSP-1 has been known to be a major activator of TGF-b1, which upregulates the TJ and P-glycoprotein of endothelial cells. Moreover, glia cell-derived neurotropic growth factor in the TGF-β family, interleukin-6, and bFGF are also involved in BNB regulation (Abbott, 2002).

Interestingly, the potential of astrocytes to induce and maintain the barrier characterisics in endothelial cells is not confined to the neural endothelial cells. The co-culture of astrocytes and nonneural endothelial cells can also induce barrier properties (Hayashi *et al*., 1997). It means that astrocytes have an intrinsic potential to play a role as a barrier supportive cell.

How are astrocytes involved in the pathology of BNB?

In the development and progression of CNS diseases (Table 1), dysfunction of the BNB is a critical event. Failure of the BNB may be a precipitating event or a consequence. For example, in traumatic injury or stroke, the BNB breakdown is a consequence of the diseases, whereas, in multiple sclerosis, it is a precipitating factor (de Vries and Dijkstra, 2004). Sometimes, the relations between dysfunction of the BNB and diseases are not definite, such as in Alzheimer's disease (Wardlaw *et al*., 2003).

Tumor. In CNS tumors, the BNB is usually impaired. The permeability of BNB increases and edema is formed around the tumor, following decrease of tight junction proteins and associated proteins, and opening of paracellular pathway.

In the developing stage of tumor, somewhat of BNB is preserved due to relatively adequate vascular endothelial growth factor (VEGF) secretion form tumor parenchyma.[56]

Table 1. CNS disorders associated with BBB dysfunction

Mechanism	CNS diseases
Neoplastic	Benign and malignant tumors in CNS
Vascular	Hemorrhage, ischemia, hypertension, vascular malformation, and etc
Edematous	Vasogenic or cytotoxic edema
Metabolic	Diabetes, toxins (heavy metals & chemicals)
Inflammatory	Multiple sclerosis, meningitis or encephalitis (viral, bacterial, fungal, & allergic)
Traumatic	Thermal injury, mechanical injury, chemical injury, irradiation, and etc
Epileptic	Seizure

With the progression of the tumor, dysfunction of the BNB aggravates to be more permeable due to variable mechanisms including overexpression of VEGF, loss of astrocytic end-foot effacement, and abnormalities of tumor vessels (Machein *et al*., 1999; Rascher *et al*., 2002).

Ischemia. Ischemia is a combined insult, the complex of loss of blood flow, oxygen, nutrients, and other molecules. Endothelial cells are so vulnerable to ischemia, resulting in genotoxic damage, disruption of tight junction proteins, and apoptosis (Mark and Davis, 2002). Astrocytes are less responsive to ischemia, for they have the anaerobic glycolysis capacity, and high antioxidant activity (Schroeter *et al*., 1999). In ischemia, astrocytes become more reactive, with the similarity to neonatal astrocytes. The function of astrocytes in the ischemia still remains unclear. Endothelial cells are less responsive to hypoxic stress when cocultured with astrocytes (Fischer *et al*., 2000). However, in a coculture under ischemia, transcellular permeability of endothelial cells increase, due to permeability factors from glial cells (Brillault *et al*., 2002).

Inflammation. Endothelial cells in the CNS are resistant to inflammation. The mature endothelial cells in the CNS limit the inflammatory response (Hickey, 2001). Nontheless, in inflammatory CNS diseases, BNB failure accompanied by tight junction disruption is well known (Petty and Lo, 2002). Many inflammatory mediators modulate the permeability of BNB (Abbott, 2000). For example, although bradykinin directly acts on endothelial cells, it can also activate NF-kB in astrocytes to release interleukin-6 (Schwaninger *et al*., 1999). Tumor necrosis factor-a produces endothelial endothelin-1 and leads to release interleukin-1b from astrocytes (Deli *et al*., 1995).

Cytokines secreted from peripheral inflammation don't usually compromise the BNB, unless accompanied by CNS pathology. Interestingly, some peripheral inflammations including cortical spreading depression (Gursoy-Ozdemir *et al*., 2004) and acute surgical pain stress (Oztas *et al*., 2004) have effects on tight junction proteins in endothelial cells.

Perspective

Recent progress of neuroscience research for molecular and cellular mechanisms of the BNB have given us great insights into understanding physiology and pathology of CNS. However, the ability to treat CNS diseases is incongruous with the progression in the neuroscience. Many CNS drugs have been discovered, but not yet successful in treating the diseases because of BNB problems.

We have learned much from fundamental research in the molecular and cellular biology of CNS. It has been known that astrocytes- endothelial cells interaction influences the BNB in both physiological and pathological conditions. However, there are still challenges. We need to clarify exact nature of BNB formation, maintenance, and disruption. We also need to bridge gaps between molecular biology, cellular biology and clinical practice. If we better understand mutual interactions between astrocytes and endothelial cells, in the near future, we could provide a critical solution to the BNB problems and create new opportunities for future success of treating CNS diseases.

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