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Review

Critical role of actin in modulating BBB permeability

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Abstract

A major obstacle in the treatment of degenerative manifestations and debilitating diseases in the central nervous system (CNS) lies in the impediment of drug delivery into these tissues. The impediment is due to a membrane barrier referred to as the blood–brain barrier (BBB). It is known that the BBB is a unique membranous structure in brain capillaries that tightly segregates the brain from systemic blood circulation. It is imperative to have a thorough understanding of the molecular components and their integrated function of this barrier to develop effective therapeutics for CNS disorders and diseases. Although there are other cell and biochemical properties that underlie this barrier function, it is well established that the barrier is mainly made up of the physical elements of tight junction (TJ) complex. The major constituents of TJ, such as occludin, claudins, zonula occludens (ZOs) and junctional adhesion molecule (JAM) have been subjects of intensive studies and reviews. However, after examining currently proposed models, we have come to believe that a cytoskeletal component-actin may play a critical role in interacting TJ molecular constituents and modulating functional TJ complex. In this review, we will discuss the correlation of temporal and spatial distribution and remodeling of actin filaments with altering integrity of TJ complexes in various systems and present a hypothesis to depict its potential role in modulating BBB permeability.

Theme: Cellular and molecular biology
Topic: Blood–brain barrier

Keywords: Blood–brain barrier; Tight junction; Actin; Cytoskeleton; Cytochalasin

Contents

1. Introduction

Despite tremendous advances in neuroscience and brain research, neurodegenerative disorders and brain tumors remain one of the leading human maladies. The major

Abbreviations: BBB, blood–brain barrier; CNS, central nervous system; JAM, junctional adhesion molecule; TJs, tight junctions; ZOs, zonula occludens proteins

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reason lies in the difficulty of administering effective treatment to manage these diseases. It is well established that a formidable membranous barrier severely hampers the access of many drugs into the brain and central nervous system (CNS) [5,62]. This permeability barrier, which comprises of the brain capillary endothelium, is known as the blood–brain barrier (BBB). Three elements underlie the BBB function: (1) a physical barrier comprised of tight junctions (TJs), which form a tight seal to intercellular diffusion, (2) the cells themselves, which exhibit a low rate of endocytosis, and (3) a metabolic barrier, consisting of specific membrane transporters expressed by endothelial cells [18,50,67]. The BBB can protect the brain from the intrusion of harmful substances. On the other hand, it presents as a major obstacle for the treatment of many CNS disorders and diseases. Therefore, a full understanding of the biochemical and ultrastructural properties of the BBB is essential in developing effective treatments for the brain and other CNS disorders. While many studies have focused on the molecular and functional delineation of the major structural constituents in TJs, such as occludin, claudin, zonula occluden (ZOs), and junctional adhesive molecule (JAM) [6,16,21,22,26,27,47,54,55,67,68,70], the role of a cytoskeletal component-actin, which represents a contractile element in the junctional endothelial cells, has attracted less attention. Although inconclusive at present, numerous studies on TJ's molecular constituents have provided ample evidence implicating the important role of actin filaments in both structural support and functional influence on the organization of TJ complex, and subsequently, modulating its junctional permeability [14,43,45,57,63,31].

2. Structural association of actin with tight junctions

It is clear that a tight junction of the BBB represents a complex junctional structure that consists of a few essential transmembrane proteins, a list of interacting cytoplasmic proteins, and cytoskeleton components, all together forming a delicate multifunctional cytoarchitecture. Through immunoprecipitation, immunogold labeling electron microscopy, and freeze fracture studies, the major interlocking transmembrane proteins, as well as their association with other cytoplasmic proteins, were gradually uncovered [3,4,6,13,20,19,21,37]. Occludin–a ~65 kDa phosphoprotein and claudins–a family of ~22 kDa proteins, are both major integral transmembrane proteins, which appear to exhibit adhesive properties and are believed to be responsible for forming a tight seal between two apposing endothelia membranes of adjacent cells [9,11,20,19,32,34,37,52,53,65]. JAM, members of the immunoglobulin superfamily, are localized at TJs of epithelial and endothelial cells [2,46,58]. JAM-1, a 32 kDa protein, has been demonstrated to associate with actin filaments at cell-to-cell contact locations [6,38,46]. It has been suggested that JAM-1, together with occludin and claudins, take part in forming the physical barrier constituents of TJs [30].

Besides possible direct interlocking by apposing extracellular protein loops of occludin, claudins, and JAM, there are other properties that share the important role of constructing a functional TJ complex. Studies have shown that ZOs and cingulin connect the integral transmembrane proteins to actin filaments [13,25,32], and dynamic regulation of perijunctional actin may function as a controlling factor in regulating paracellular permeability [1,41]. The study of Haskins et al. has also demonstrated an essential role of ZOs in developing and stabilizing TJs by directly interacting with actin filaments [25,70]. In addition, cingulin, a double-stranded myosin-like protein, has been proposed to be a functionally important constituent, linking ZOs of TJs to actin filaments [13]. The above information and other descriptions from a few structural models regarding TJ complex and BBB permeability [22,54,67] suggest that actin may be more than a simple structural component of tight junction. In fact, there are ample ultrastructural data that implicate the temporal expression, dynamic organization, and spatial distribution of the actin cytoskeleton in altering TJ complex under various conditions [14,17,40,42,45,31]. Therefore, actin is likely to play a critical role in modulating the integrity of TJs.

3. Functional association of actin with tight junctions

Although not in the brain vascular endothelial system, early studies on intestinal absorptive cells led by Madara et al. first provided evidence suggesting that the barrier function of tight junctions was regulated through its association with actin filaments [43]. They demonstrated the influence of a cytokine-interferon-γ on the TJ protein constituents and structural alterations [41,42]. It clearly showed the redistribution of ZOs as well as the occludin molecules due to INF-γ. The altered distribution coincided with perturbed paracellular permeability, which was evidenced by decreased transepithelial resistance. Furthermore, it showed that disorganization of actin filaments occurred concurrently with increased paracellular permeability. Youakim and Ahdieh also addressed similar observations of concurrent disruptions of ZOs expression and apical actin filaments followed by increased paracellular permeability in the study [73]. These disruptions were induced on the intestinal epithelial cell with interferon-γ treatment. They also noted that occludin appeared to show little change. While occludin may be an important component of tight junction structure [24], the data in this study suggested that occludin did not function as a major regulator to the paracellular permeability. Rather, the study suggested a direct connection between the state of actin organization and the integrity of paracellular permeability. Unfortunately, this study did not mention another important component of tight junction—claudin. It would be very interesting to see the state of claudin under the same

parameters in their study. However, it should be noted that in a study by Mark and Davis on perturbations of BBB permeability in an ischemia–hypoxia model, they showed that claudin was not involved in the hypoxia-induced paracellular permeability changes; instead, their data suggested the involvement of occludin in the alteration of paracellular permeability [45]. Other studies by Madara et al. also led to speculation that ZOs may be directly involved in the mechanistic regulation of barrier function by modulating actin filaments with its TJ complex [41].

In the brain microvascular system, Crawford et al. have shown that reduction of oxygen supply (hypoxia stress) as well as post-hypoxia reoxygenation could induce alterations of actin distribution in brain microvascular endothelial cells [14]. In vivo, hypoxia is known to cause damage to the BBB permeability that can lead to the development of cerebral edema. Primary culture of brain microvascular endothelial cells has been used as an in vitro BBB model to evaluate the mechanisms by which hypoxia regulates paracellular permeability. Mark and Davis' in vitro studies showed that, after exposure to hypoxic stress, the metabolic and physical barrier of brain microvascular endothelial cells was perturbed and resulted in an increase of paracellular permeability in brain microvascular endothelial cells [45]. In this study, they further showed that changes in paracellular permeability after hypoxic insult exhibited direct correlation with alterations in expression of actin filaments [45]. Crawford et al. clearly showed that reoxygenation, following a period of hypoxia, induced rapid polymerization of actin filaments, and concurrent with rapid polymerization, spatial redistribution of actin filaments was also observed. More importantly, the thickening of the cortical actin coincided with the reduction of junctional permeability [14]. A similar observation was demonstrated in the study by Mark and Davis' [45]. Therefore, in addition to the role of structural support, actin reorganization appears to contribute a direct role in the formation and maintenance of the integrity of TJ complexes.

4. Structural perturbation of tight junctions induced by actin-disrupting agents and extracellular mediators

Agents such as cytochalasin B, cytochalasin D, and phalloidin have traditionally been used to disrupt polymerization of actin filaments and thus are useful tools in studies correlating actin reorganization with perturbations of TJs, both structurally and functionally [7,43,63]. In studies of intestinal epithelial cells, direct association of perijunctional actin with TJ has been identified by transmission electron microscopy [29,40]. Treatment of intestinal epithelial cells with cytochalasin D leads to an increase in sodium permeability and a decrease in transepithelial resistance [43]. This study showed that perturbation of intracellular permeability and transepithelial resistance may be caused by substantial structural disruption between actin filaments and occluding TJs. In the study using Madin–Darby canine kidney (MDCK) epithelial cells, Meza et al. showed that both transepithelial resistance and actin distribution were affected by cytochalasin B simultaneously [49]. Furthermore, decreased transepithelial resistance and altered actin distribution were reversed after removal of the disrupting agent. These data suggested an intimate association between the distribution of actin filaments and TJ permeability [49]. This finding was consistent with that of a similar study using cytochalasin D treatment on MDCK epithelial cells by Stevenson and Begg [63]. In the study of epithelial restitution after single-cell defect, the closure and sealing of TJs were inhibited due to perturbations of actin polymerization in the presence of cytochalasin D. It further showed that perturbations of actin polymerization by actin-disrupting agents were reversible following removal of these agents [17]. These studies reinforce the notion that actin may play a critical role in modulating physiological functions of TJs. However, the precise molecular mechanism of how actin exerts its influence on TJs is currently unknown. Nevertheless, since inhibition of actin polymerization and altered distribution of actin filaments invariably induce the defect in the functional TJ complex, without the apparent change of occludin or claudin, it is tempting for us to speculate that the intact and normal distribution of actin filaments is required to anchor and enforce the close interlocking of occludin and claudin molecules between apposing endothelia membranes. It is well known that dynamic actin filaments provide the basic infrastructure for maintaining cell morphology and various functions [39]. Actin-dependent cell shape change can exert its regulatory control during cell growth and differentiation, such as during angiogenesis [31] or osteogenic differentiation [60]. The studies of Ingber et al. showed that actin cytoskeleton dependent cell spreading was critical for progression of the endothelial cell cycle [31]. Cells respond to various stimuli and extracellular messengers, which results in the remodeling or reorganization of the actin cytoskeleton. For example, human lens epithelial cells respond to growth factors, leading to the reorganization of their actin cytoskeleton [44]. This response is thought to play a critical role in lens epithelial cell proliferation, migration, elongation, and survival. In response to thrombin, human umbilical endothelial cells showed cell shape changes and gap formation in the monolayer that was contributed by the reorganization of actin microfilaments [36]. Extracellular messenger mediated actin-cytoskeletal remodeling has been implicated in the maintenance or regulation of various epithelial and endothelial paracellular permeability [8,44,69]. Brown and Davis suggested in their recent study that calcium influx might modify occludin expression and subsequently alter the TJ function. However, although the alteration of actin structure was evident in that study, the direct correlation between calcium influx and altered actin organization was not established [10]. In our view, it is also possible that actin remodeling may affect the secondary messengers, such as Ca+2 mobilization, and subsequently affect the calcium mediated enzyme activity, which in turn mediates the modification, expression,

and distribution of ZOs, occludin, and claudin at the junctional complexes. In fact, Korkiamaki et al. showed that intact actin-cytoskeleton was crucial for calcium mobilization in keratinocytes [35]. The mechanism to delineate our hypothesis is speculative at present. However, in many cases stated above, actin appears to play an active role in regulating cellular functions rather than acting as a static component of cellular structure. In this review, we suggest a simple model in Fig. 1 to emphasize the active role of these intact actin filaments in regulating the integrity of tight junction complex. In our hypothesis, the adhesive interactions between apposing occludin and claudins may become secondary or supplementary components in maintaining a functional TJ complex. This different point of view on BBB regulation may serve to partially explain why neither occludin nor claudin appeared to be essential in maintaining TJ integrity in several studies, including gene knockout models [4,27,73,24,61]. This hypothesis may have vital implications in the development of drug delivery into the CNS, as it could lead to important discoveries in facilitating drug transport across the BBB. This hypothesis awaits future studies.

5. Future directions for studies on modulating BBB permeability

In light of the importance of a thorough understanding of TJs, as well as BBB permeability regulations, much more work needs to be done to delineate the precise roles and contributions of individual structural components in the TJ complex.

There are many excellent reviews on the subject of TJ molecular organization and its functional significance in various epithelial and endothelial systems [22,48,51,45,66,67,72]. In this review, we focused on the endothelial cell system, particularly TJs in the BBB. The development, composition, and regulation of TJs within the BBB are highly complex but essential for maintaining a healthy microenvironment in the CNS. Any minor defect or imbalance of the integrity of this barrier is known to result in serious maladies. On the other hand, the ability to create reversible and desirable permeability in this barrier is key in introducing effective remedies for many CNS disorders and diseases. Numerous approaches are currently under intense study to overcome impediment and to facilitate drug delivery across this formidable membranous barrier. These include: rational drug design, i.e. modification of the drug itself, coupling of the drug to a vector for receptor-mediated or adsorption-mediated transcytosis, and artificial disruption of the BBB permeability [18,50,64]. There are advantages and disadvantages for each of the approaches. For example, microencapsulated immuno-liposome, as well as PEGylated liposome, delivery holds promising results in enhancing efficacy and transport of peptide or protein drugs across the BBB through the receptor-mediated process; however, high cost and extremely complex manufacturing procedures may

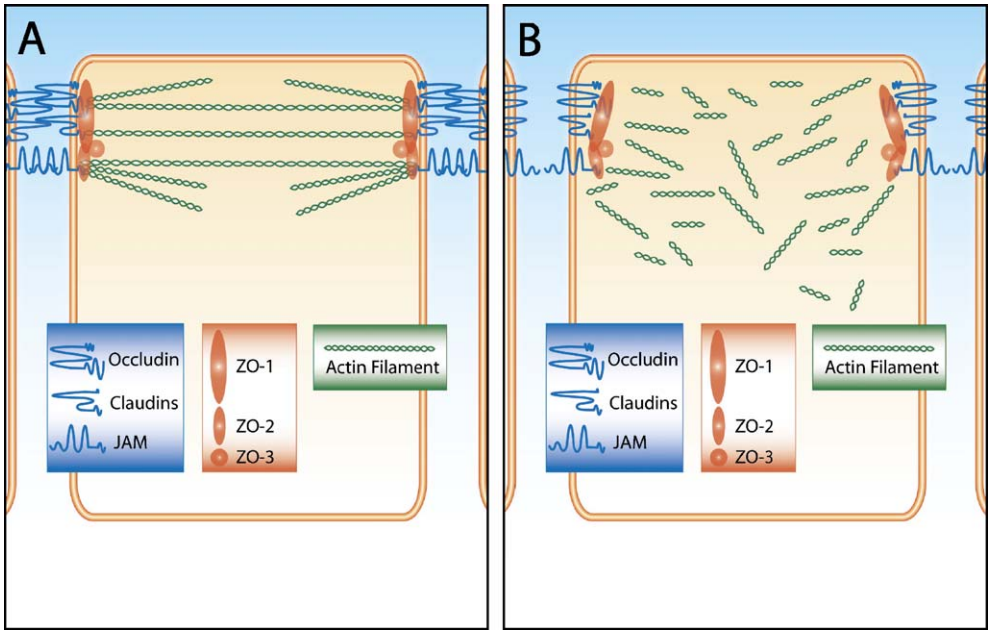

Fig. 1. Simplified diagram to depict the active role of actin in modulating BBB permeability. Only major protein constituents, such as occludin, claudin, zonula occluden (ZOs), and junctional adhesion molecule (JAM) are presented in this simplified endothelial cell tight junction (TJ) complex. The emphasis is directed to the active role of actin filament reorganization in modulating the TJ complex. Diagram A represents the cell under normal conditions. The intact actin filaments exert an anchoring force to stabilize the occludin and claudin transmembrane proteins against both sides of the lateral cell membranes, and the extracellular loops of the transmembrane proteins protrude into the pericellular space to maintain the normal tight seal of the TJ complex. Diagram B shows the disruption of actin filaments in the presence of cytochalasin D treatment. It should be noted that, without the anchoring force exerted by the intact actin filaments, the protruding extracellular loops of the occludin and claudins proteins are slightly withdrawn from the pericellular space, and thus the junctional tight seal is loosened.

raise serious practical concerns. In addition, potential steric hindrances between the large carrier and the small active drug partner, as well as drug distribution, may require further evaluations. Any other vector or receptor-conjugated drug designs face the same potential problems. Using reagents to induce transient BBB permeability is another strategy to enhance drug delivery. For example, biochemical disrupting agents such as vasoactive leukotrienes, bradykinin, and histamine have shown certain aspects of clinical success [12,15]. However, there are certain adverse effects, such as brain edema and other undesirable damages. Although TJ permeability disruption, caused by histamine, is known to be associated with the reorganization actin filaments [28,71], none of the above approaches for overcoming the barrier is directed to investigate the important function of actin organization in modulating TJ integrity. We presented our viewpoints in this article to raise attention to the potentially vital role of actin in this respect. There are further questions that need to be addressed. How is the polymerization or organization of actin filaments regulated by extracellular signal(s)? How is this signal transduction pathway relayed to modulate the functional TJ complex? How is the functional TJ complex modulated in various physiological or pathological conditions? The family of Rho protein family GTPase, such as RhoA, Rac1, and Cdc42, are believed to be dynamic regulators of actin filaments [23,33,56,57,59]. How are perturbations of actin reorganization related to this GTPase regulated signal pathway? These are all pertinent questions that require future attention to better understand the molecular mechanisms involved in the regulation of functional TJ complexes. The understanding is essential in the search for better strategies in managing CNS disorders and diseases. In this regard, we would further suggest specifically that, by ways of manipulating the reorganization of actin filaments, one may find alternatives to accomplishing the goal of safe disruption of BBB permeability. By that, we may reach the ultimate objective of developing more effective methods for drug delivery into the central nervous system.

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