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BRAIN RESEARCH

Research Report

Influence of basolateral condition on the regulation of brain microvascular endothelial tight junction properties and barrier function

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ABSTRACT

Basolateral condition of the brain microvascular endothelium is believed to influence blood–brain barrier (BBB) phenotype, although the precise transcriptional and post-translational mechanisms involved are poorly defined. *In vivo*, the basolateral surface of the blood–brain endothelium is bathed in serum-free interstitial fluid and encompassed by astrocytic end-feet. We hypothesized that these conditions impact on BBB function by directly modulating expression and biochemical properties of tight junctions. To investigate this, an *in vitro* transwell culture model was employed to selectively modify the basolateral environment of bovine brain microvascular endothelial cells (BBMvECs). In the absence of basolateral (but not apical) serum, we observed higher levels of expression, association and plasma membrane localization for the tight junction proteins, occludin and zonula occludens-1 (ZO-1), in parallel with elevated transendothelial electrical resistance (TEER) and reduced ^{14}[C]-sucrose permeability of BBMvEC monolayers. We further examined the effects of non-contact co-culture with basolateral astrocytes (C6 glioma) on indices of BBMvEC barrier function in both the presence and absence of serum. Astrocyte co-culture with serum led to enhanced occludin protein expression, occludin/ZO-1 association, and ZO-1 membrane localization, in parallel with increased TEER of BBMvEC monolayers. Astrocyte co-culture in the absence of serum (i.e. basolateral conditions most consistent with *in vivo* BBB physiology) however, gave the highest increases in BBMvEC barrier indices. Thus, we can conclude that factors influencing condition of the basolateral environment of the brain microvasculature can directly, and independently, modify BBB properties by regulating the expression and biochemical properties of the tight junction proteins, occludin and ZO-1.

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Abbreviations: BBB, Blood–Brain Barrier; BBMvEC, Bovine Brain Microvascular Endothelial Cell; CNS, Central Nervous System; FCS, Fetal Calf Serum; FGF, Fibroblast Growth Factor; GAPDH, Glyceraldehyde Phosphate Dehydrogenase; IP, Immunoprecipitation; ISF, Interstitial Fluid; JAM, Junctional Adhesion Molecule; SF, Serum Free; SEM, Standard Error of the Mean; TEE, Trans Endothelial Exchange; TEER, Trans Endothelial Electrical Resistance; ZO-1, Zonula Occludens-1

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1. Introduction

The blood–brain barrier functions to maintain homeostasis of the central nervous system (CNS) by preventing potentially harmful blood-borne solutes entering the brain micro-environment. *In-vivo*, this barrier exhibits high TEER and is impermeable to charged particles, proteins, ions, hydrophilic molecules and hormones that could act as neurotransmitters (Tanobe et al., 2003). Consistent with its protective role, disruption of BBB integrity leads to vascular leakage, a central pathophysiologic mechanism of many diseases including ischemic injury and stroke (Fredriksson et al., 1987; Brown and Davis, 2002), multiple sclerosis (Williams et al., 1994), meningitis/encephalitis (Tunkel and Scheld, 1993) and neurodegenerative diseases (Mattila et al., 1994; Poland et al., 1995).

BBB phenotype involves a continuum of specialized inter-endothelial protein junction complexes, which form an effective seal to prevent paracellular solute diffusion. Barrier function is physically attributed to the tight junction (Balda and Matter, 1998; Rubin and Staddon, 1999), although the adherens junction is also functionally important in this context (Brown and Davis, 2002; Farkas et al., 2005). Tight junctions comprise occludin, junctional adhesion molecules 1–3 (JAMs), claudins, cingulin and the zonula occludens family members (ZO-1/2/3), the latter responsible for anchoring the junctional complex to the actin cytoskeleton (Anderson and Van Itallie, 1995; Balda and Matter, 1998).

Several physiological parameters are known to regulate BBB integrity in a polar-specific manner. Publications have reported reduced TEER and increased paracellular permeability when the basolateral surface of the brain microvascular endothelium, ordinarily exposed to serum-free interstitial fluid (ISF) *in vivo*, is exposed to serum (Nitz et al., 2003; Lohmann et al., 2004; Hoheisel et al., 1998). Interestingly, this polar-specific effect has also been reported for epithelial cells (Marmorstein et al., 1992; Chang et al., 1997). Astrocytes located basolaterally to the endothelium have also been implicated in the up-regulation of BBB function (Jeliazkova-Mecheva and Bobilya, 2003; Gee and Keller, 2005; Haselhoff et al., 2005). The signaling pathways mediating such polar-specific effects are only partially defined however, as are the transcriptional and post-translational mechanisms involved. We therefore hypothesized that these basolateral conditions impact on BBB phenotype by directly modulating the expression and biochemical properties of tight junction proteins. To investigate this hypothesis, we have employed a transwell cell culture model to examine how basolateral conditioning of low passage BBMvECs by serum and astrocytes, examined alone and in combination, potentially modulates the expression and biochemical properties of occludin and ZO-1, in parallel with consequences for BBB integrity.

2. Results

Using low passage BBMvECs cultured in Transwell®-Clear inserts, the experimental approach taken in these studies involved: (i) removal of basolateral serum and (ii) inclusion of basolateral astrocytes. Relative to the *in vivo* situation therefore, the starting condition (i.e. FCS) is non-physiological, whilst the applied experimental condition (i.e. SF and/or inclusion of astrocytes) is physiological. Thus, the data can be interpreted as the impact of injury-vs-health on endothelial barrier function.

2.1. *Serum-dependent modulation of BBMvEC barrier function: Occludin and ZO-1 levels*

Occludin and ZO-1 mRNA and protein levels were monitored following 24 h culture of BBMvECs in the presence or absence of basolateral serum. In the absence of serum, occludin mRNA levels increased by 1.62±0.09 fold, concomitant with a 1.93±0.23 fold increase in protein levels (Fig. 1a). Furthermore, in the absence of serum there was no statistically significant change in ZO-1 mRNA levels (Fig. 1a), although ZO-1 protein levels increased by 1.76±0.18 (Fig. 1b).

2.2. *Serum-dependent modulation of BBMvEC barrier function: Occludin/ZO-1 association and localization*

Protein–protein association of occludin and ZO-1 was monitored following 24 h incubation of BBMvECs in the presence or absence of basolateral serum. In the absence of serum, the level of occludin detected in anti-ZO-1 immunoprecipitates increased by 2.86±0.69 (Fig. 2a). Subcellular localization of occludin and ZO-1 was also monitored by immunocytochemistry. In the presence of serum, no membrane localization of occludin was observed, whilst ZO-1 immunoreactivity along the cell border appeared highly discontinuous (Fig. 2bi–ii). In the absence of serum, immunoreactivity of both proteins became appreciably more concentrated and continuous along the cell–cell border (Fig. 2biii–iv). No localization changes were observed when serum was removed from the apical compartment, confirming polar-specificity of these effects.

2.3. *Serum-dependent modulation of BBMvEC barrier function: Permeability and TEER*

Transendothelial permeability of BBMvEC monolayers to ^{14}[C]-sucrose was assessed following 24 h culture of BBMvECs in the presence or absence of basolateral serum. In the absence of serum, permeability decreased significantly from 10.5±0.6% to 8.2±0.8% trans endothelial exchange (TEE) after the 60 min test period (Fig. 3a). Barrier integrity was also monitored by TEER. In the absence of serum, TEER increased by 32.3±13.8% (Fig. 3b).

2.4. *Astrocyte co-culture in the presence of serum: Impact on BBMvEC barrier function*

BBMvEC barrier function was monitored following 24 h culture of BBMvECs in the absence or presence of co-cultured astrocytes (i.e. C6 glioma *with* basolateral serum). In the presence of co-cultured astrocytes, we observed appreciably enhanced ZO-1 immunoreactivity along the cell–cell border (Fig. 4ai–ii). In parallel with this observation, astrocyte co-culture also resulted in a 37±4.0% increase in TEER (Fig. 4b).