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Blood–brain barrier opening to large molecules does not imply blood–brain barrier opening to small ions

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ABSTRACT

Neuroimaging of exogenous tracer extravasation has become the technique of choice in preclinical and clinical studies of blood–brain barrier permeability. Such tracers have a larger molecular weight than small ions, neurotransmitters and many drugs. Therefore, it is assumed that tracer extravasation indicates both permeability to these and the cancelation of the electrical polarization across the barrier. Electrophysiological anomalies following intracarotideal administration of dehydrocholate, a bile salt causing extravasation of the albumin-binding tracer Evans blue, seemingly supported this. By contrast, electron microscopic studies suggested a different hierarchical pattern of blood–brain barrier dysfunction, a milder degree of impairment being characterized by increased function of the transcellular pathway and a severe degree by opening of the tight junctions. This would imply that the extravasation of macromolecules can occur before disruption of the electrical barrier. However, functional evidence for this has been lacking. Here, we further investigated the electrophysiological anomalies following intracarotideal application of dehydrocholate in rats and found that it caused focal cerebral ischemia by middle cerebral artery thrombosis, the electrophysiological recordings being characteristic of long-lasting spreading depolarization. These observations indicated that intracarotideal dehydrocholate is not a suitable model to study the isolated dysfunction of the blood–brain barrier. Second, we studied the topical application of dehydrocholate to the brain and the application of mannitol into the carotid artery. In both models, we found significant extravasation of Evans blue but no changes in either extracellular potassium or the CO_2-dependent intracortical direct current deflection. The latter is assumed to depend on the proton gradient across the barrier in rats which we confirmed in additional experiments *in vivo* and *in vitro*. The stability of the extracellular potassium concentration and the CO_2-dependent direct current deflection are two functional tests which indicate the integrity of the electrical barrier. Hence, our results provide functional evidence that the blood–brain barrier opening to large molecules does not necessarily imply the opening to small ions consistent with the hierarchy of damage in the previous electron microscopic studies.

Introduction

In 1885, Paul Ehrlich showed the lack in permeability of brain vessels to dyes, and, in 1900, Max Lewandowsky postulated a barrier between blood and neuronal tissue, the so called blood–brain barrier (BBB) (Ehrlich, 1885; Lewandowsky, 1900). Under physiological conditions, the restrictive nature of the BBB hinders most polar solutes and macromolecules larger than 400 to 500 Da to enter the brain's extracellular space (Fig. 1).

It is often assumed that the transfer of hydrophilic molecules across the dysfunctional BBB largely depends on their size. The basis of this concept is that the dysfunction of the BBB occurs on the level of the junctional complexes of which disruption is indeed characterized by a size-dependent paracellular passage of molecules into the brain as shown in experiments with homozygous claudin-5 knockout mice (Nitta et al., 2003). This concept implies that BBB opening to macromolecules such as albumin is associated with opening to small ions such as potassium and protons. Experiments with the bile salt sodium dehydrocholate (DHC) seemingly supported this. When injected into the carotid artery, DHC caused extravasation of the albumin-binding dye Evans blue (Spigelman et al., 1983), and, electrophysiological recordings revealed marked persistent changes in the direct current (DC) potential associated with drastically diminished, subsequent CO_2-dependent DC deflections (Nita et al., 2004).

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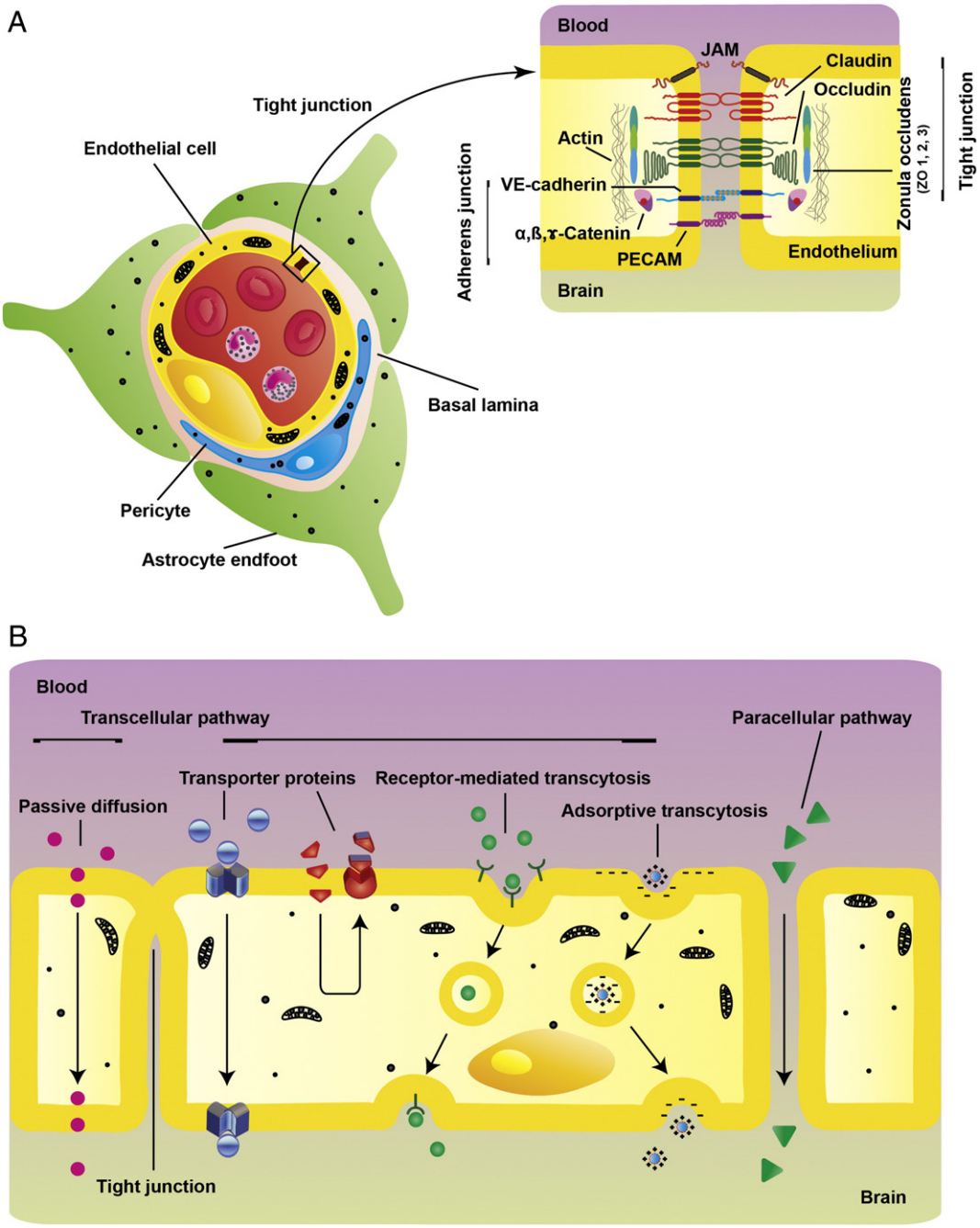

Fig. 1. Anatomical structure of the BBB. (A) The BBB is characterized by endothelial cells that have no fenestrae and minimal transcytotic vesicles but mitochondria in abundance. They line the inner luminal surface of the vessels and are fastened together by continuous, belt-like endothelial cell–cell complexes of adherens and tight junctions (inset). The tight junctions are composed of a complex of proteins, including transmembrane proteins (claudins and occludins), and zonula occludens proteins (ZO 1, 2, and 3), which bind the transmembrane proteins to the actin/myosin cytoskeleton. The junctional adhesion molecules (JAM) are members of the immunoglobulin superfamily and appear to act as cell-adhesion molecules for leukocytes (Abbott et al., 2010). The adherens junctions include vascular endothelial cadherin (VE-cadherin) which support a structural maintenance of the BBB and the formation of tight junctions and catenins that link cadherins to skeleton proteins as well as platelet–endothelial cell adherens molecule (PECAM). Additional cellular elements of the BBB are pericytes which share with the endothelial cells a common capillary basement membrane and astrocytic foot processes which cover the capillaries (Abbott et al., 2006; Beard et al., 2011; Neuwelt et al., 2011). (B) While the paracellular pathway is normally blocked by the junctional complexes, the trafficking of essential solutes across the BBB either into or out of the brain parenchyma occurs by the transcellular pathway, mediated and controlled through specific transport or carrier molecules in a highly selective fashion. Dysfunction of the BBB occurs in many brain diseases, including stroke, epilepsy, trauma, infectious and degenerative disorders (Abbott et al., 2006; Cornford and Oldendorf, 1986; Korn et al., 2005; Neuwelt et al., 2011; Stanimirovic and Friedman, 2012; Tomkins et al., 2001). While it has long been recognized that BBB dysfunction is associated with brain diseases, only recently the BBB has been suggested as active player in epileptogenesis and neurodegeneration (Ivens et al., 2007; Marchi et al., 2007; Seiffert et al., 2004; Tomkins et al., 2007; van Vliet et al., 2007).

These electrophysiological anomalies were regarded as a consequence of BBB opening to small ions, canceling the electrical polarization across the BBB. Normally, the electrical polarization produces a resting potential difference of ~4 mV between intravascular and interstitial compartment (Revest et al., 1994).

Hyperosmolar agents such as mannitol are another experimental tool causing dysfunction of the BBB. Mannitol was indeed shown to cause functional changes in key components of the junctional complexes such as β-catenin which could lead to the junctions' opening (Farkas et al., 2005). However, previous electron microscopic studies