The capillaries of the brain are particularly special, as they are not simply conduits for blood, but are primarily responsible to ensure that the neurons function in a strictly regulated homeostatic interstitium. Brain endothelial cells (BECs) express a plethora of ion channels on its luminal and abluminal surfaces, namely: potassium (K⁺) channels (i.e., Kir2 and Kv1), chloride (Cl⁻)/bicarbonate (HCO₃⁻) channels, as well as a number of ion-solute exchangers (Redzic et al., 2011). These channels essentially prioritize vectorial transendothelial transport, especially for the regulation of K⁺ flux across the blood-brain barrier (BBB) (Redzic et al., 2011). The differences between the K⁺ concentration of the brain interstitium and plasma is only 2 mM to 4 mM, but the maintenance of this ionic concentration difference provides a constancy for the neuronal resting membrane potential, their associated firing thresholds and the preservation of a constant level of neuronal excitability. The stability of the interstitial environment surrounding the brain’s neurons is the foundational essence of our persona; it is the basis for the continuity of our making of intellectual decisions and the stability of our psychological essence. Furthermore, pathologies emanating from paracellular (PC) tight junction (TJ) permeability have been implicated in psychiatric disorders, epilepsy, multiple sclerosis, neuroinflammation, stroke and cardiovascular disease (Greene et al., 2019). Thus, the regulation of permeability across the BBB is of interest from a physiological, psychological and clinical perspective.

The brain capillary: The cerebrovascular microvasculature is comprised of specialized endothelial cells, responsible for modulating the constituents of the brain’s interstitial fluid (ISF), mitigating against chemical instability in the neuronal milieu. These cells are contiguous, interconnected by TJ protein complexes at their apical membranes which in essence provides the occlusion of the PC spaces, restricting the flux of solutes and ions to transendothelial transport at the apical and basal membrane domains (Gunzel and Yu, 2013). In fact, the TJs are mandatory in conferring the characteristic establishment of one of the most impermeable epithelia (> 1000–3000 Ohm cm²) (Rajagopal et al., 2019), which, in turn, reflects on the ‘tightness’ of the PC space. Any relaxation or compromise of the PC impermeability would counter the most impermeable epithelia (> 1000–3000 Ohm cm²) (Rajagopal et al., 2019), which, in turn, reflects on the ‘tightness’ of the PC space. Any relaxation or compromise of the PC impermeability would counter the

Are claudin-5 tight-junction proteins in the blood-brain barrier porous? The recent theoretical findings are largely based on the work of Yu et al. (2009), who conducted molecular fluorescence and electrophysiological experimentation on TJ pore formation. They investigated the porosity of claudin-2 inserted into Madin-Darby Canine Kidney cells by demonstrating increased conductance and permeability and also used “conductance scanning” to deduce pore-formation in claudin-2 TJ proteins. Others have used various experimental models of knockout-mice to deduce the importance of TJs to the homeostatic regulation of the brain. Claudin-5 is a tetraspanning protein, with two extracellular loops (ECLs), ECL 1 and 2, and two cytoplasmic domains of its protein which largely influences BBB resistance/impermeability. However, the ECL1 in claudin-5 possesses two cysteines which function in PC tightening and its ECL2 does not have a pore-forming function, but rather a narrowing and holding function (Haseloff et al., 2015).

Given the historical and robust impermeability of the BBB, the prevailing view is that claudin-5 bestows upon the BECs’ PC spaces a high level of ionic and solute impermeability (Figure 1A).

TJ pore-forming proteins: TJ pore-formation is largely due to claudin mutations caused by charged residues of its ECLs or the addition of cysine’s covalent modification which are reported to alter PC permeability (Rajagopal et al., 2019).

The nature of the TJ pore is theoretically envisaged as, firstly, being involved in passive processes driven by electrochemical gradients that have been generated through active transcellular transport mechanisms. Secondly, mechanisms to regulate the directional fluxes are absent, viz., it does not rectify or behave as membrane-based channels. Given the variety of claudins (1–30) and the characteristic bouquet of claudins which define the various epithelia utilizing TJs, it is probable that each epithelia may require a non-regulated passage for the movement of ions or water via the PC space. A simple analogy may involve the active transport of Na⁺ across an epithelium, which creates the electrochemical gradient to drive Cl⁻ through the PC route which has a set of claudin-based TJs which specifically only allow Cl⁻ anion permeability (Gunzel and Yu, 2013).

The case for paracellular ionic impermeability in the brain: Given the highly restrictive character of the BBB, its strict homeostatic role to correctly regulate the ionic milieu (K⁺, Na⁺, Cl⁻) channels, as well as the maintenance of the highly restrictive character of the BBB, its strict homeostatic role to correctly regulate the ionic milieu (K⁺, Na⁺, Cl⁻), ensuring consistency in neuronal function, it is difficult to reconcile ionic-pore-like features in the claudin-5 based TJ of BECs. In fact, there is little to no experimental data to convincingly support the premise that this is indeed a feature of the current experimental and theoretical understanding of the normative BBB. Instead, a PC pathway in the BBB which is porous to ions would suggest a PC-shunt that would make ionic transcellular regulation quite inefficient. Given the scarcity of supporting experimental evidence, and the lack of theoretical imperative for an ionic claudin-5 pore for the BBB, we caution against the mathematical and computational postulates which are not based on a clearly defined physiological position, as it has the potential to steer research down the proverbial cul-de-sac. This is the case for Rajagopal et al. (2019) who has postulated numerous mathematical and computational configurations for ionic pores through the claudin-5 TJ.

A role for aquaporosity in claudin-5? It is conspicuous that in vivo BECs are not known to express aquaporins (AQPs). Endorsing this view, Dolman et al. (2005) have shown that primary rat BEC cultures do not express AQP1 after the third passage. Furthermore, in the absence of co-cultured astrocytes, this expression is suppressed. This supports the view that under normal physiological conditions in vivo BECs do not express AQP1, or any other AQP (Dolman et al., 2005; Francesca and Rezzani, 2010; Papadopoulos and Verkman, 2013). The main function of AQPs is to facilitate the movement of water in response to osmotic gradients and, therefore, it begs the question: just how does water cross the capillary endothelium of the brain? Although it has been mooted that water crosses the brain endothelium via the PC pathway, across the TJ, claudin-5, driven by osmotic and chemical gradients. This postulate is supported by the elegant mathematical and computational postulates which allow for the elucidation of an experimental phenomenon.

Water homeostasis in neural tissue: Surrounding the endothelial cells of brain capillaries, the perivascular astrocyte end-foot processes abundantly express AQP4. These foot-processes are joined to each other via permeable junctional complexes and essentially envelopes the brain capillaries, forming the perivascular space (Figure 1B). By extension, the strongly expressed AQP4 on these processes, suggests a role for astrocytes in regulating water homeostasis of the brain’s ISF (Francesca
Furthermore, the concept that BEC TJs have pore-forming functions will directly impact the existing theoretical understanding of BBB permeability, and it also becomes an additional factor implicating the etiopathology of neurodegenerative disorders (Greene et al., 2019). We, therefore, caution against postulates that have no grounding in established physiological or experimental supporting evidence. Given this principle, and the absence of AQPs in the in vivo brain capillary endothelium, we postulate water may indeed flow through the PC pathways, via the ‘theoretical aqua-pores of claudin-5’ (Figure 1B). This postulate is further supported by the current evidence that the perivascular astrocytic foot-processes possess high levels of AQP4 with implications for water homeostasis of the brain ISF. Furthermore, the pharmacokinetics involved in the treatment of the central nervous system still requires extensive investigation, due to the restrictive nature of BBB interaction (Upadhyay, 2014). It remains a rate-limiting factor when designing neuropharmaceuticals. Thus, the regulation of the permeability across the BBB remains an area requiring intense experimental scrutiny.

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